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(54) Interferon gamma inducing factor

(57) Disclosed are created stable polypeptides which are capable of inducing the production of interferon-gamma by immunocompetent cells. The present polypeptides contain specific amino acid sequences usually derived from the wild-type polypeptides, being capable of the production of interferon-gamma, by replacing the cysteine(s) with different amino acid(s). The present polypeptides possess a stability and an activity of inducing the production of IFN-γ by immunocompetent cells, both of which are significantly higher than

those of the wild-type polypeptides. In addition to the activity, the present polypeptides can exhibit remarkable activities of inducing the formation of killer cells and enhancing thier cytotoxicities. The present polypeptides are easily obtainable by the process according to the present invention using recombinant DNA techniques. Thus the present polypeptides are useful for agents to treat and/or prevent susceptive diseases such as viral diseases, infections, malignant tumors, and immunopathics.

Description

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The present invention relates to novel biologically active polypeptides, more particularly, artificially created polypeptides which are commonly capable of inducing the production of interferon-gamma (hereinafter abbreviated as "IFN- γ ") by immunocompetent cells.

The present inventors successfully isolated some polypeptides which are capable of inducing the production of IFN-y by immunocompetent cells, as well as cloned cDNAs which encode the polypeptides (hereinafter called "the wild-type polypeptides"); the relating inventions are disclosed in Japanese Patent Kokai Nos.27,189/96 and 193,098/96 and Japanese Patent Application No.67,434/96. It is known that the wild-type polypeptides usually contain SEQ ID NOs:1-3 as consensus partial amino acid sequences, and that they usually possess properties of inducing the formation of killer cells and enhancing their cytotoxicities, in addition to the property of inducing production of IFN-γ, a useful biologically active protein. Because of the properties, the use of the wild-type polypeptides as antiviral, antimicrobial, antitumor, and/or anti-immunopathic agents, etc. is in great expectation.

However, as described in Japanese Patent Application No.67,434/96 by the present applicant, there is the problem that natural cells commonly do not enable producing the wild-type polypeptide in desired amounts. The present inventors energetically investigated the cause, revealing that the wild-type polypeptides usually exist in the form of precursor exhibiting no biological activity in natural cells. The precursor has been proved to be converted into an active form by the action of converting enzymes such as interleukin-1β converting enzymes, which is described in Japanese Patent Application Nos.207,691/96 and 213,267/96 by the present applicant.

The wild-type polypeptides are probably instable, which would be involved in the above problem as another cause. Progress in recombinant DNA techniques of recent years have facilitated to remove and/or replace one or more amino acids in biologically active proteins to develop mutagenized polypeptides. However, even the progressed recombinant DNA techniques couldn't improve the stability of every protein with the inherent activity, unless taking trails and errors on the proteins individually.

In view of the foregoing, the first object of the present invention is to provide a polypeptide with significantly improved stability, while substantially retaining a biological activity of the wild-type polypeptide.

The second object of the present invention is to provide a process for producing the polypeptide.

The third object of the present invention is to provide a use of the polypeptide for a pharmaceuticals.

The present inventors energetically studied to attain the above objects, revealing that a polypeptide is more stable than the wild-type polypeptide, wherein the stale polypeptide contain an amino acid sequence derived either from a polypeptide containing the partial amino acid sequences of SEQ ID NOs:1-3 by replacing one or more of the cysteines with a different amino acid(s), or from the cysteine-replaced amino acid sequences by adding, removing and/or replacing one or more amino acids to and/or at position(s) excepting the position(s) where the cysteine(s) has been replaced; and that some of the stable polypeptides, in which the cysteine(s) have been replaced, exhibit an activity of inducing the production of IFN-γ by immunocompetent cells significantly higher than the wild-type polypeptides. These polypeptides proved to be easily produced in a desired amount by using recombinant DNA techniques and to exhibit less toxicities. Based on the above, the present polypeptides were confirmed to be effectively used not only as an IFN-γ inducer but also as a pharmaceutical.

The first object of the present invention is attainable by an isolated polypeptide which is capable of inducing the production of interferon-gamma by immunocompetent cells, said polypeptide containing either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs:1-3 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine.

The second object of the present invention is attainable by a process for producing a polypeptide, which comprises the steps of culturing a cell containing a DNA encoding the present polypeptide to produce a polypeptide, and collecting the produced polypeptide from the resulting culture.

The third object of the present invention is attainable by an agent for susceptive diseases, which contains the present polypeptide as an effective ingredient.

FIG. 1 is the restriction map of a recombinant DNA "pCSHIGIF/MUT12" encoding a polypeptide according to the present invention.

FIG. 2 is the restriction map of a recombinant DNA "pCSHIGIF/WT" encoding the wild-type polypeptide from human origin.

FIG. 3 shows the time course of activity upon incubation of the polypeptides according to the present invention and the wild-type polypeptide, from human origin.

FIG. 4 is the restriction map of a recombinant DNA "pCSHIGIF/MUT21" encoding another polypeptide according to the present invention.

FIG. 5 is the restriction map of a recombinant DNA "pCSHIGIF/MUT25" encoding further another polypeptide according to the present invention.

- FIG. 6 is the restriction map of a recombinant DNA "pCSHIGIF/MUT32" encoding further another polypeptide according to the present invention.
- FIG. 7 is the restriction map of a recombinant DNA "pCSHIGIF/MUT41" encoding further another polypeptide according to the present invention.
- FIG. 8 is the restriction map of a recombinant DNA "pCSHIGIF/MUT35" encoding further another polypeptide according to the present invention.
- FIG. 9 is the restriction map of a recombinant DNA "pCSHIGIF/MUT42" encoding further another polypeptide according to the present invention.
- FIG. 10 is the restriction map of a recombinant DNA "pCSMIGIF/MUT11" encoding further another polypeptide according to the present invention.
- FIG. 11 is the restriction map of a recombinant DNA "pCSMIGIF/WT" encoding the wild-type polypeptide from mouse origin.
- FIG. 12 shows the time course of activity upon incubation of the polypeptides according to the present invention and the wild-type polypeptide, from mouse origin.
- FIG. 13 is the restriction map of a recombinant DNA "pCSMIGIF/MUT12" encoding further another polypeptide according to the present invention.

[Explanation of Symbols]

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The symbol "CMV" indicates a cytomegalovirus promoter.

The symbol "IFNss" indicates a nucleotide sequence encoding the signal peptide of the subtype α 2b of human interferon- α .

The symbols "IGIF/WT" and "mIGIF/WT" indicate cDNAs encoding any one of the wild-type polypeptides.

The symbols of "IGIF/MUT12", "IGIF/MUT21", "IGIF/MUT25", "IGIF/MUT32", "IGIF/MUT32", "IGIF/MUT41", "IGIF/MUT35", "IGIF/MUT42", "mIGIF/MUT11" and "mIGIF/MUT12" indicate cDNAs each of which encodes one of the polypeptides according to the present invention.

The followings are preferred embodiments according to the present invention. The polypeptides according to the present invention include all of the polypeptides which is capable of inducing production of interferon-gamma by immunocompetent cells, wherein said polypeptides contain either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs:1-3 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine. The different amino acids to replace the cysteine(s) are not restricted to any types, as far as the resulting polypeptide, containing an amino acid sequence replaced with the different amino acid(s), exhibits an activity of inducing production of IFN-y by immunocompetent cells in the presence or absence of an appropriate cofactor, as the wild-type polypeptides containing SEQ ID NOs:1-3 as consensus partial amino acid sequences, and a stability significantly higher than that of the wild-type polypeptides. The different amino acids include serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine, among which the most preferable amino acid is serine or alanine. Embodiments of the amino acid sequences, containing SEQ ID NOs:1-3 as consensus partial amino acid sequences, in which one or more cysteines are to be replaced with different amino acid(s) are the wild-type polypeptides containing the SEQ ID NO:4 or 5. The SEQ ID NO:4 contains cysteines at the 38th, 68th, 76th, and 127th positions from the N-terminus. The SEQ ID NO:5 contains cysteines at the 7th, 75th, and 125th positions.

The present polypeptides include those containing the amino acid sequence of any one of SEQ ID NOs:6-12, which are derived from the wild-type polypeptide containing SEQ ID NO:4, those containing the amino acid sequence of SEQ ID NO:13 or 14, which are derived from the wild-type polypeptide containing the amino acid sequence of SEQ ID NO:5, and those containing an amino acid sequence derived from any one of SEQ ID NOs:6-14 by adding, removing, and/or replacing one or more amino acids to and/or at position(s) excepting the positions where the cysteine(s) have been replaced while retaining the desired biological activities and stability. The wording "one or more amino acids" means the number of amino acids which conventional methods such as site-directed mutagenesis can usually add, remove or replace. The polypeptides containing any one of SEQ ID NOs:6-14 possess both stability and biological activities significantly higher than those of the wild-type polypeptides.

The present polypeptides can be produced by recombinant DNA techniques of: transforming appropriate host cells with DNAs encoding the present polypeptides to obtain a cell containing the DNAs, culturing the cells containing the DNAs to produce the polypeptides, and collecting the produced polypeptides from the resulting culture. The present invention additionally provides a process using the recombinant DNA techniques for producing the present polypeptides, by which the present polypeptides can be easily obtained in a desired amount.

The DNAs used in the present process include all of the DNAs encoding any one of the present polypeptides, which can be obtained by a method of either artificial mutagenesis of DNAs from natural sources or chemical synthesis.

An example of the former method is as follows: preparing a DNA with the nucleotide sequence of SEQ ID NO:25 or 28 encoding the amino acid sequence of SEQ ID NO:4 or 5, respectively, from a natural cell as a source, and then applying "overlap extension", a method reported in Robert M. Horton et al. *Methods in Enzymology*, Vol.217 (New York: Academic Press, Inc., 1993), pp.270-279, to the DNA to replace one or more codons for the cysteines in SEQ ID NO: 4 or 5 with codon(s) for different amino acid(s). The present DNAs include DNAs containing any one of the nucleotide sequence of SEQ ID NOs:15-21, derived from SEQ ID NO:25, SEQ ID NOs:22 and 23, derived from SEQ ID NO:28, the complementary nucleotide sequences to SEQ ID NOs:15-23, and others derived from these nucleotide sequences by replacing one or more of the nucleotides with different one(s) without altering the amino acid sequences encoded thereby. An example of the latter method is chemical synthesis, by which the present DNAs are obtainable in usual manner based on the nucleotide sequences of SEQ ID NOs:9-15. Once obtained by any method, the present DNAs can be easily amplified to a desired amount by using PCR.

Generally in this field, when allowing a DNA encoding a polypeptide to express in a host cell, to improve the expressing efficiency or the biological activities of the polypeptide expressed, one or more nucleotides in the DNA can be replaced with different ones, and an appropriate promoter(s) and/or enhancer(s) can be linked to the DNA. The present DNAs can be also altered similarly as such. For example, nucleotide sequences for recognition sites by appropriate restriction enzymes, initiation codons, termination codons, and/or appropriate signal peptides including the signal peptide of the subtype a2b of interferon-α, shown in SEQ ID NO:16, can be arbitrary linked to the 5'- and/or 3'-termini of any of the nucleotide sequences of SEQ ID NOs:9-15, unless the resulting polypeptides diminish the desired biological activities and stabilities.

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The present DNAs can express the present polypeptides with improved stabilities and biological activities after introduced into appropriate host cells from microbial, vegetal, or animal origin, preferably, animal origin. The present DNAs can be introduced into the host cells in the form of recombinant DNAs. The recombinant DNAs usually comprise one of the present DNAs and one of autonomously replicable vectors, which are obtainable by conventional recombinant DNA techniques, once the present DNAs are obtained. Embodiments of the vectors into which the present DNAs can be inserted are plasmid vectors including pCD, pCDL-SRα, pKY4, pCDM8, pCEV4 and pME18S, which usually comprise nucleotide sequences suitable for expressing the present DNAs in hosts, e.g., promotors, enhancers, replication origins, terminators of transcription, splicing sequences, and/or selection markers. The use of vectors with a promotor such as a heat shock protein promotor or the interferon-α promotor disclosed by the present applicant in Japanese Patent Kokai No.163,368, enables to regulate the present DNAs expression in the transformats by external stimuli.

To insert the present DNAs into the vectors, any conventional method in this field can be used. For example, DNAs containing the present DNAs and the vectors as above are digested by restriction enzymes and/or ultrasonication before the resulting fragments from the present DNAs are ligated with the vector fragments. Digestion by the restriction enzymes, which act on specific nucleotides, preferably, Accl, BamHI, Bg/II, BstXI, EcoRI, HindIII, Notl, PstI, Sacl, Sall, Smal, Spel, Xbal, Xhol, etc., facilitate to ligate the DNA fragments with the vector fragments. When ligating the DNA fragments with the vector fragments, they are, if necessary, first annealed, and then treated with a DNA ligase in vivo or in vitro. The recombinant DNAs thus obtained can be unlimitedly replicated in hosts from microbial or animal origin.

The recombinant DNAs can be introduced into host cells suitable to produce the present polypeptides. Whereas any cells conventionally used as host cells in this field can be used in the present invention, the host cells from yeast or mammalian origin are more preferable when the polypeptides produced are used for pharmaceuticals. Embodiments of the host cells from mammalian origin are epithelial, interstitial, and hemopoietic cells from human, monkey, mouse, and hamster, which include 3T3 cells, C127 cells, CHO cells, CV-1 cells, COS cells, HeLa cells, MOP cells, and their mutants. To introduce the present DNAs into the hosts, any conventional methods can be used, e.g., DEAE-dextran method, calcium phosphate transfection method, electroporation method, lipofection method, microinjection method, and viral infection method as using retrovirus, adenovirus, herpesvirus, and vaccinia virus, etc. To select clones producing the present polypeptides from the transformants, the transformants can be cultured before examining the resulting cultures for the present polypeptides produced. The recombinant DNA techniques using mammalian host cells are detailed in publications such as Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA eds., *Jikken-Igaku-Bessatsu, Saibo-Kogaku Handbook* (The handbook for the cell engineering), (Tokyo, Japan: Yodosha. Co., Ltd., 1992), and Takashi YOKOTA and Kenichi ARAI eds., *Jikken-Igaku-Bessatsu, Biomanual Series 3, Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), (Tokyo, Japan: Yodosha Co., Ltd., 1993).

The transformants thus obtained, cells containing the present DNAs, can produce the present polypeptides intracellularly and/or extracellularly when cultured in culture media. For the culture media, any conventional ones used for transformants can be used. The culture media generally comprise: buffers as a base; inorganic ions such as sodium ion, potassium ion, calcium ion, phosphoric ion, and chloric ion; micronutrients, carbon sources, nitrogen sources, amino acids and vitamins, which can be used depending on metabolic abilities of the cells; and sera, hormones, cell growth factors, and cell adhesion factors, which are used if necessary. Examples of the culture media are 199 medium, DMEM medium, Ham's F12 medium, IMDM medium, MCDB 104 medium, MCDB 153 medium, MEM medium, RD

medium, RITC 80-7 medium, RPMI-1630 medium, RPMI-1640 medium, and WAJC 404 medium. Culturing the present transformants under the following conditions can generate cultures containing the present polypeptides: inoculating the present transformants into the culture media to give a cell density of 1 x 10^4 - 1 x 10^7 cells/ml, more preferably, 1 x 10^5 - 1 x 10^6 cells/ml, and culturing the cells in suspension- or monolayer-cultures at about 37°C for 1-7 days, more preferably, 2-4 days, if necessary, while replacing the culture media with fresh ones. The cultures thus obtained usually contain the present polypeptides in a concentration of about 1-100 μ g/ml, which may vary depending on the types of the transformants or culture conditions used.

While the cultures thus obtained can be used intact as an IFN-y inducer, they can be usually subjected to the steps for purifying the present polypeptides before use, following the steps of separating the present polypeptides from the cells or the cell debris by filtration, centrifugation, etc., and, if necessary, which may follow a step for disrupting the cells by ultrasonication, cell-lytic enzymes, and/or detergents. To purify the present polypeptides, conventional techniques in this field for purifying biologically active polypeptides can be arbitrary used, e.g., salting-out, dialysis, filtration, concentration, fractional precipitation, ion-exchange chromatography, gel filtration chromatography, adsorption chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis and/or isoelectric focusing gel electrophoresis. The present polypeptides thus purified can be concentrated and/or lyophilized into liquids or solids depending on final uses. The monoclonal antibodies disclosed in Japanese Patent Application No.58,240/95 by the present applicant are extremely useful to purify the present polypeptides. Immunoaffinity chromatography using the antibodies can minimize the costs and the labors for obtaining the present polypeptides with a relatively high purity.

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The present polypeptides can be usually added to media for culturing immunocompetent cells to produce INF- γ , or administered to humans to treat or prevent INF- γ susceptive diseases. In the case of producing IFN- γ , lymphocytes separated from mammalian peripheral bloods or established cell lines such as HBL-38 cells, Mo cells ATCC CRL8066, Jurkat cells ATCC CRL8163, HuT78 cells ATCC TIB161, EL4 cells ATCC TUB39, L12-R4 cells, and mutant strains thereof are suspended in culture media containing 0.1 ng - 1 μ g/ml, preferably, 1 - 100 ng/ml of the present polypeptides. Then, the cells are cultured by conventional methods for about 1-100 hours, if necessary, in the presence of T-cell stimulating agents such as mitogens, interleukin 2, and anti-CD3 antibodies, and while replacing the culture media with fresh ones. To collect the IFN- γ produced, the resulting cultures can be subjected to technique(s) appropriately selected from those conventional for purifying INF- γ , e.g., salting-out, dialysis, filtration, concentration, fractional precipitation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, adsorption chromatography. affinity chromatography, isoelectric focusing chromatography, gel electrophoresis, and isoelectric focusing gel electrophoresis. etc.

Since the present polypeptides induce production of IFN- γ , agents for susceptive diseases containing the present polypeptides as an effective ingredient can induce production of IFN- γ in human bodies when administered to human, and can treat and/or prevent IFN- γ -susceptive diseases. When the present polypeptides have activities of enhancing cytotoxicities and/or inducing formation of killer cells such as NK cells, LAK cells (lymphokine-activated killer cells), and cytotoxic T cells, besides the IFN- γ inducing activity, as in Examples of the present invention, described below, the killer cells are also involved in treating and/or preventing susceptive diseases. Thus, the wording "susceptive diseases" as referred to in the present invention includes all of the diseases which can be treated and/or prevented by the direct or indirect action of IFN- γ and/or killer cells. The susceptive diseases are viral diseases including hepatitis, herpes, condyloma, and AIDS; infections including candidiasis, malaria, cryptococcoses, diseases caused by Yersinia infection, and tuberculosis; solid malignant tumors including renal carcinoma, mycosis fungoides, and chronic granulomatous diseases; blood-cell-derived malignant tumors including adult T cell leukemia, chronic myelogenous leukemia, and malignant lymphoma; immunopathies including allergies, rheumatism, and collagen diseases; and osteoporosis, etc. The present agents additionally containing interleukin 3 can completely treat or remit leukopenia and thrombopenia caused by radiation therapy or chemotherapy in treating malignant tumors, in addition to leukemia and myeloma.

Thus the present agents for susceptive diseases can be widely used for treating and/or preventing the aforesaid susceptive diseases in the forms of an antitumor agent, an antiviral agent, an antiseptic, an anti-immunopathic agent, a platelet-proliferating agent, and a leukocyte-proliferating agent, etc. The present agents can be usually processed into a liquid, paste, or solid form, containing 0.000001 - 100 w/w %, preferably, 0.0001 - 0.1 w/w % of the present polypeptides on a dry solid basis, while the form and the contents may vary depending on the uses and on the types and the symptoms of diseases to be treated and/or prevented.

The present agents can contain not only the present polypeptides solely but also other physiologically acceptable agents to form compositions, e.g., carriers, excipients, diluents, biological response modifiers and stabilizers, and if necessary, one or more other biologically active substances. The stabilizers can be proteins including serum albumins, and gelatins, saccharides including glucose, fructose, sucrose, maltose, lactose, trehalose, sorbitol, mannitol, maltitol, and lactitol, and buffers with phosphoric acid and/or succinic acid. Embodiments of the other biologically active substances are interferon-α, interferon-β, interferon-γ, interferon-β, interferon-β, granulo-

cyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, carboquone, cyclophosphamide, aclarubicin, thiotepa, busulfan, anbitabine, cytarabine, 5-fluorouracil, 5-fluoro-1-(tetrahydro-2-furyl)uracil, methotrexate, actinomycin D, chromomycin A3, daunorubicin, doxorubicin, bleomycin, mitomycin C, vincristine, vinblastine, L-asparaginase, radio gold colloidal, Krestin®, picibanil, lentinan, and Maruyama vaccine.

Among the above agents, those containing interleukin 2 are particularly useful because the interleukin 2 effects as a cofactor when the present polypeptides induce production of IFN- γ by immunocompetent cells. Thus the agents, additionally containing a natural or recombinant interleukin 2, can induce production of IFN- γ in a desired level by even immunocompetent cells that scarcely produce IFN- γ by the single action of present polypeptides.

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The present agents additionally containing interleukin 12 can induce IFN-γ in a particularly high level which the present polypeptides or interleukin 12 per se cannot achieve. In addition, since the present polypeptides can enhance the inhibitory action of interleukin 12 on production of immunoglobulin E antibodies, the present agents with interleukin 12 are useful as an anti-immunopathic agent to treat and/or prevent atopic diseases such as atopic asthma, atopic bronchial asthma, hay fever, allergic rhinitis, atopic dermatitis, vascular edema, and atopic dyspepsia. Because there occasionally exists interleukin 12 in human bodies while in a relatively-low level, then the present polypeptides can achieve the desired effects in the human bodies alone.

The present agents include those in a unit of dose form, which means a physically separated and formed medicament suitable for administration, and contains the polypeptides required for a daily dose or in a dose from 1/40 to several folds (up to 4 folds) of the daily dose. Embodiments of such medicaments are injections, liquids, powders, granules, tablets, capsules, sublinguals, ophthalmic solutions, nasal drops and suppositories.

The present agents can be used for administering orally or parenterally to patients and for activating antitumor cells *in vitro* as described below, both of which effect to treat and/or prevent the susceptive diseases. For example, the present agents are usually administered orally to patients or parenterally to patients' intradermal tissues, subcutaneous tissues, muscles or veins as observing the patients' symptoms and recuperations at a dose in the range of about 0.1 - 50 mg/shot, preferably, one μ g/shot to one mg/shot of the present polypeptides 1-4 times/day or 1-5 times/week for one day or one year.

The present agents can be also useful in so called "antitumor immunotherapies" using interleukin 2. The antitumor immunotherapies are generally classified into (i) a method administering the interleukin 2 directly to the bodies of patients with malignant tumors, and (ii) a method introducing antitumor cells activated by the interleukin 2 ex vivo to the patients (adoptive immunotherapy), any of which can exert significantly improved effects when used with the present polypeptides. For example, in the method (i), the present polypeptides can be administered to patients at an dose ranging from about 0.1 µg/shot/adult to one mg/shot/adult one to ten times simultaneously with or before the interleukin 2 administration. The dose of interleukin 2, which may vary depending on the types of the malignant tumors, the patients' symptoms and the dose of the present polypeptides, is usually in the range of 10,000 - 1,000,000 units/shot/adult. In the method (ii), to the media for culturing mononuclear cells or lymphocytes collected from patients with malignant tumors in the presence of the interleukin 2, the present polypeptides can be usually added with an amount of about 0.1 ng - 1 μg per 1×106 of the cell. After the cells are cultured for a prescribed period of time, NK cells or LAK cells are collected from the resulting cultures to be returned to the patients' bodies. Diseases as targets for the present antitumor immunotherapies are: solid malignant tumors such as colonic cancer, rectal cancer, gastric cancer, thyroid carcinoma, cancer of tongues, bladder carcinoma, choriocarcinoma, hepatoma, prostatic cancer, carcinoma uteri, laryngeal, lung cancer, breast cancer, malignant melanoma, Kaposi's sarcoma, cerebral tumor, neuroblastoma, tumor of ovaries, testicular tumor, osteosarcoma, cancer of pancreas, renal cancer, hypernephroma, and hemangioendothelioma; and blood cell malignant tumors such as leukemia and malignant lymphoma, etc.

The present DNAs, encoding the present polypeptides, are also useful in so called "gene therapies". According to conventional techniques in the gene therapies, the present DNAs can be introduced into patients with IFN-γ- and/or killer cell-susceptive diseases by direct injection after inserted into vectors derived from viruses such as retrovirus, adenovirus and adeno-associated virus, or after incorporated into cationic- or membrane fusible-liposomes. Alternatively, the present DNAs can be introduced into the patients by self-transplanting lymphocytes which have been collected from the patients before the DNAs have been introduced into. In adoptive immunotherapies with the gene therapies, the present DNAs can be introduced into effector cells similarly as using the conventional techniques. This can enhance cytotoxicities of the effector cells to tumor cells, resulting in improvement of the adoptive immunotherapy. In tumor vaccine therapy with the gene therapies, tumor cells from patients, into which the present DNAs can be introduced similarly as above, are self-transplanted after proliferated *ex vivo* up to give a desired cell number. The transplanted tumor cells act as vaccines in the patients to exert a improved antitumor immunity specific to the antigens. Thus the present DNAs exhibit remarkable effects in the gene therapies for diseases including viral diseases, microbial diseases, malignant tumors, and immunopathies. General procedures for the gene therapies are detailed in Takashi SHIMADA, Izumi SAITO and Keiya OZAWA eds., *Jikken-Igaku-Bessatsu*, *Biomanual UP Series*, *Idenshichiryo-no-Kisogijutsu* (Basic techniques for the gene therapy), (Tokyo, Japan: Yodosha Co., Ltd., 1996).

The following examples explain the present invention: Examples A-1 to A-9 describe preferred embodiments of

the polypeptides and the process for producing thereof according to the present invention, and Examples B-1 to B-5 describe the preferred embodiments of the agents for susceptive diseases according to the present invention. The techniques in Examples A-1 to A-9 are conventional ones used in this field, which are detailed in publications, e.g., Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA eds., *Jikken-Igaku-Bessatsu*, *Saibo-Kogaku Handbook* (The handbook for the cell engineering), (Tokyo, Japan: Yodosha. Co., Ltd., 1992), and Takashi YOKOTA and Kenichi ARAI eds., *Jikken-Igaku-Bessatsu*, *Biomanual Series 3*, *Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), (Tokyo, Japan: Yodosha Co., Ltd., 1993).

Example A-1

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Production of polypeptide

Example A-1(a)

15 Construction of recombinant DNA

Genomic DNA was collected by conventional manner from BALL-1 cells, ATCC RCB0256, an established cell line derived from human acute lymphocytic leukemia, and oligonucleotides with the nucleotide sequences of 5'-ACACCTC-GAGCCACCATGGCCTTGACCTTTGCTTTAAC-3' as a sense primer (the sense primer 1) and of 5'-TTGCCAAAG-TAGCCCACAGAGCAGCTTG-3' as an antisense primer (the antisense primer 1) were chemically synthesized based on the nucleotide sequence for the signal peptide of the subtype α2b of human interferon-α, shown in SEQ ID NO:24, described in K. Henco et al. *Journal of Molecular Biology,* Vol.185, pp.227-260 (1985). In a 0.5 ml-reaction tube, one μg of the genomic DNA, 10μl of 10 × PCR buffer, one μl of 25 mM dNTP mix, and adequate amounts of the sense primer 1 and the antisense primer 1 were mixed, and sterilized distilled water was added to the mixture to give a volume of 99 μl. To the mixture, one μl of 2.5 units/μl Pfu DNA polymerase was further added. The mixture was subjected to 30 cycles of incubations at 94°C, 600C, and 72°C for one minute, respectively, to perform a PCR, resulting in obtaining a DNA fragment (the DNA fragment 1) which comprised the nucleotide sequence of SEQ ID NO:24, a site recognized by a restriction enzyme of *Xho*I, linked to the 5'-terminus of the SEQ ID NO:24, and a sequence of 1st-11th nucleotides in SEQ ID NO:25, linked to the 3'-terminus of the SEQ ID NO:24.

The recombinant DNA "pHIGIF", containing the nucleotide sequence of SEQ ID NO:25 encoding the wild-type polypeptide with the amino acid sequence of SEQ ID NO:4, was prepared according to the methods described in Japanese Patent Kokai No.193,098/96 by the present applicant. The wild type polypeptide, with the amino acid sequence of SEQ ID NO:4, contains partial amino acid sequences of SEQ ID NOs:1-3 in the regions of 16th-21st, 30th-35th, and 51st-55th amino acids. Oligonucleotides with the nucleotide sequences of 5'-CTGCTCTGTGGGCTACTTT-GGCAAGCTTGAATC-3' as a sense primer (the sense primer 2) and 5'-ACACGCGGCCGCCTAGTCTTCGTTTT-GAACAG-3' as an antisense primer (the antisense primer 2) were chemically synthesized in usual manner based on SEQ ID NOs:25 and 26. In a 0.5 ml-reaction tube, one ng of the recombinant DNA "pHIGIF", 10µl of 10 × PCR buffer, one µl of 25 mM dNTP mix and adequate amounts of the sense primer 2 and the antisense primer 2 were mixed, and then sterilized distilled water was added to the mixture to give a volume of 99µl. To the mixture, one µl of 2.5 units/µl Pfu DNA polymerase was further added. The mixture was subjected to 30 cycles of incubations at 94°C, 60°C and 72°C for one minute, respectively, to perform a PCR, resulting in obtaining a DNA fragment (the DNA fragment 2) which comprised the nucleotide sequence of SEQ ID NO:25, a termination codon of 5'-TAG-3' and a site recognized by a restriction enzyme of *Not*I, linked to the 5'-terminus of the SEQ ID NO:25, and a sequence of 57th-69th nucleotides in SEQ ID NO:24, linked to the 3'-terminus of the SEQ ID NO:25.

In a 0.5 ml-reaction tube, one ng of the DNA fragments 1 and 2 each, $10 \,\mu$ l of $10 \times PCR$ buffer, and one μ l of 25 mM dNTP mix were mixed, and sterilized distilled water was added to the mixture to give a volume of 99μ l. The mixture was incubated at 94° C for 3 minutes and slowly cooled to 37° C, and incubated at the temperature for 15 minutes. The mixture was given one μ l of 2.5 units/ μ l Pfu DNA polymerase and slowly heated to 72° C, and then incubated at the temperature for 2 minutes. After added adequate amounts of the sense primer 1 and the antisense primer 2, the mixture was subjected to 30 cycles of incubations at 940C for one minute, at 60° C for one minute, and at 72° C for 30 seconds, to perform a PCR, resulting in obtaining a DNA fragment (the DNA fragment 3) which comprised the nucleotide sequence of SEQ ID NO:26.

An oligonucleotide with the nucleotide sequence of 5'-CTCTGTGAAGTCTGAGAAAATTTCAACTC-3', as a mutagenic sense primer to replace the 283rd nucleotide of guanine in SEQ ID NO:26 with a cytosine, was chemically synthesized by usual manner. A PCR was performed similarly as that for obtaining the DNA fragment 1, but using the DNA fragment 3 as a template and the mutagenic sense primer for the sense primer 1. The PCR resulted in obtaining a DNA fragment (the DNA fragment 4) which comprised a nucleotides sequence identical to 276th-570th nucleotides in SEQ ID NO:26 except for the 287th nucleotide replaced with a cytosine.

An oligonucleotide with the nucleotide sequence of 5'-GAGTTGAAATTTTCTCAGACTTCACAGAG-3', as a mutagenic antisense primer to replace the 287th nucleotide of guanine in SEQ ID NO:26 with a cytosine, was chemically synthesized by usual manner. A PCR was performed similarly as that for obtaining the DNA fragment 2, but using the DNA fragment 3 as a template and the mutagenic antisense primer for the antisense primer 1. The PCR resulted in obtaining a DNA fragment (the DNA fragment 5) which comprised a nucleotides sequence identical to Ist-304th nucleotides in SEQ ID NO:26 except for the 287th nucleotide replaced with a cytosine.

A PCR was performed similarly as that for obtaining the DNA fragment 3, but using the DNA fragments 4 and 5 as templates, to obtain a DNA fragment (the DNA fragment 6) containing a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:6. The DNA fragment 6 comprised the nucleotide sequence of SEQ ID NO:15, the nucleotide sequence of SEQ ID NO:24 and a site recognized by a restriction enzyme *Xho*I, linked to the 5'-terminus of the SEQ ID NO:15, and a termination codon of the nucleotides of 5'-TAG-3' and a site recognized by a restriction enzyme *Not*I, linked to 3'-terminus of the SEQ ID NO:15.

After the DNA fragment 6 by restriction enzymes *Xho*l and *Not*l was cleaved to generate a DNA fragment of 555 bps, 25 ng of the generated DNA fragment was mixed with 10 ng of a plasmid vector "pCDM8", commercialized by Invitrogen Corporation, San Diego, USA, which had been cleaved by the *Xho*l and *Not*l, and then the mixture was incubated at 16°C for 30 minutes using a ligation kit "LIGATION KIT VERSION 2", commercialized by Takara Shuzo Co., Tokyo, Japan. By cloning, an autonomously replicable recombinant DNA "pCSHIGIF/MUT12" consisting of 4,494bp was obtained. As shown in FIG. 1, in the recombinant DNA "pCSHIGIF/MUT12", a cDNA "IGIF/MUT12" with the nucleotide sequence of SEQ ID NO:15 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α. As shown in the accompanied amino acid sequence, the nucleotide sequence of SEQ ID NO:15 encodes the amino acid sequence of SEQ ID NO:6, derived from the wild-type polypeptide with SEQ ID NO:4 by replacing the cysteine at the 68th position.

For a control, an autonomously replicable recombinant DNA "pCSHIGIF/WT" was prepared similarly as above excepting the DNA fragment 6 replaced with the DNA fragment 3. As shown in FIG. 2, in the recombinant DNA "pCSHIGIF/WT", a cDNA "IGIF/WT" with the nucleotide sequence of SEQ ID NO:25, encoding the wild-type polypeptide, was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of subtype α2b of human interferon-α.

Example A-1(b)

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Production of polypeptide by transformant

The recombinant DNA "pCSHIGIF/MUT12", obtained in Example A-1(a), was introduced by conventional competent-cell method into an *Escherichia coli* strain "MC1061/P3", commercialized by Invitrogen Corporation, San Diego, USA, to obtain a transformant. The transformant was cultured in L medium (pH 7.2) containing 20 μg/ml ampicillin and 10 μg/ml tetracycline at 37°C for 18 hours under shaking conditions. The resulting culture was centrifuged to separate the cells, and the separated cells were subjected to conventional alkali-SDS method to extract the recombinant DNA.

2.5 ml of DME medium (pH 7.4) supplemented with 10 v/v % fetal bovine serum was put into each well of six-well microplates, and 1.8×10⁵ cells of COS-1, ATCC CRL1650, an established cell line derived from African green monkey kidney, was inoculated into each well. The microplates were incubated at 370C for 24 hours in a 5 v/v % CO2 incubator. After the incubation, the media were removed, and the wells were washed with DME medium containing 50 mM Tris-HCl buffer (pH 7.4). To each well, 1.8 ml of DME medium containing 2.8 µg/ml of the recombinant DNA obtained above, 50 mM Tris-HCl buffer (pH 7.4), 0.4 mg/ml DEAE-dextran and 0.1 mM chloroquine was added, and the microplates were incubated at 37°C for 4 hours in a 5 v/v % CO₂ incubator. After the incubation, the media were removed, and 2.5 ml of 10 mM phosphate buffer (pH 7.4) containing 10 v/v % dimethylsulfoxide and 140 mM NaCl was added to each well, and then the microplates were stood at ambient temperature for 2 minutes. After the standing, the buffers were removed, and the wells were washed with DME medium containing 50 mM Tris-HCl buffer (pH 7.4). To each well, 2.5 ml of a culture medium "COS MEDIUM", commercialized by COSMOBIO Co., Ltd., Tokyo, Japan, was added, and the microplates were incubated at 37°C for 3 days in a 5 v/v % CO2 incubator to culture the cells. The resulting culture was analyzed by Western blotting using the monoclonal antibody described in Japanese Patent Kokai No.231,598/96. The analysis proved that the present polypeptide, capable of inducing production of IFN-γ by immunocompetent cells and containing an amino acid sequence derived from SEQ ID NO:4 by replacing the cysteine at 68th position with a serine, was produced in the culture in an amount of about 20 ng/ml.

As a control experiment, the recombinant DNA "pCSHIGIF/WT" obtained in Experiment A-1(a) was treated similarly as the recombinant DNA "pCSHIGIF/MUT12". Consequently, the wild-type polypeptide capable of inducing production of IFN- γ was produced in the culture in an amount of about one ng/ml. This yield was no more than 5% of that obtained by using the recombinant DNA "pCDHIGIF/MUT12". This indicates that the polypeptide according to the present invention, in this Example, is more stable and exhibits biological activities higher than the wild-type polypeptide.

Example A-1(c)

Purification of polypeptide

The culture containing the present polypeptide that was obtained in Experiment A-1(b) was centrifuged to collect a supernatant. After the supernatant was fed to a column, which was packed with a gel for immunoaffinity chromatography using the monoclonal antibody, prepared according to the methods disclosed in Japanese Patent Kokai No. 231,598/96 by the present applicant, and preliminarily washed with phosphate-buffered saline (hereinafter abbreviated as "PBS"), a fresh PBS was run through the column to wash, and then 0.1 M glycine-HCl buffer (pH 2.5) containing one M NaCl was run to elute. From the eluted fractions, those containing the polypeptide capable of inducing production IFN-γ by immunocompetent cells were collected. The collected fractions were dialyzed against PBS at 40C for 18 hours, and then concentrated by membrane-filtration followed by lyophilization to obtain a solid polypeptide with a purity of about 95 % or higher and a recovery of about 50 % to the culture of the starting material. In parallel, the culture containing the wild-type polypeptide, obtained by using the recombinant DNA "pCSHIGIF/WT", was purified similarly as above for a control in analyzing the physicochemical properties as described below.

Example A-1(d)

Molecular weight

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SDS-Polyacrylamide gel electrophoresis of the polypeptide in Example A-1(c) in the presence of 2 w/v % dithiothreitol as a reducing agent, according to the method described in U. K. Laemli, *Nature*, Vol.227, pp.680-685 (1970), exhibited a main band of a protein capable of inducing IFN- γ at a position corresponding to a molecular weight of about 18,000-19,500 daltons. The molecular weight makers used were bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (30,000 daltons), soy bean trypsin inhibitor (20,100 daltons), and α -lactoalbumin (14,000 daltons). Example A-1(e)

N-Terminal amino acid sequence

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Conventional analysis using a protein sequencer "MODEL 473A", commercialized by Perkin-Elmer Corp., Norwalk, USA, revealed that the polypeptide in Example A-1(c) had the amino acid sequence of SEQ ID NO:27 in the N-terminal region. Example A-1(f)

Stability

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The present polypeptide or the wild-type polypeptide, in Example A-1(c), was dissolved in a culture medium "COS MEDIUM", commercialized by COSMOBIO Co., Ltd., Tokyo, Japan, to give about 10 ng/ml, and the solution was incubated at 40°C for 24 hours. After 0, 0.5, 1, 2, 4, 6, 8, 12, or 24 hours from starting the incubation, a portion of each solution was sampled. The samples were individually assayed on IFN-γ inducing activity, according to the methods described below, in Example A-1(g), to study the time course of the activity upon the incubation. Percentage (%) of the residual activity at every point was calculated based on the activity at the starting point. The results are in FIG. 3. As shown in FIG. 3, the polypeptide in this Example was more stable and retained the activity longer than the wild-

type polypeptide. This evidences that the amino acid replacement used in this Example can effectively improve the

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Example A-1(g)

Production of IFN-y by immunocompetent cells

stability of the wild type polypeptide without reducing the biological activities.

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KG-1 cells, ATCC CCL246, an established cell line derived from human acute myelogenous leukemia, were inoculated into RPMI-1640 medium (pH 7.4) with no sera to give a density of 3×10⁵ cells/ml and cultured at 37°C for 4 days in a 5 v/v % CO₂ incubator. The cultured cells were collected and suspended to give a density of 3×10⁶ cells/ml in RPMI-1640 medium (pH 7.4) supplemented with 10 v/v % fetal bovine serum. 0.1 ml of the cell suspension was put into each well of 96-well microplates, and 0.1 ml of a solution containing the present polypeptide or the wild-type polypeptide, obtained in Example A-1(c), which had been diluted appropriately, was added to each well. Thereafter, the cells were cultured at 37°C for 24 hours in a 10 v/v % CO₂ incubator. 0.1 ml of supernatants of the cultures were collected from the wells and examined on productions of IFN-γ by conventional enzyme-immunoassay. As a blank, an experiment was taken in parallel identically as above but using no polypeptides. Table 1 shows the results. The pro-

ductions of IFN- γ in Table 1 were expressed with international units (IU), calculated based on the IFN- γ standard Gg23-901-530, obtained from the International Institute of Health, USA.

Table 1

Polypeptide concentration, ng/ml	Production of IFN-γ, IU/ml*
0	0 (0)
0.1	0.7 (0.6)
0.2	3.0 (2.4)
0.4	8.1 (7.4)
0.8	20.0 (18.9)
1.0	30.0 (25.9)

^{*)} Value in parentheses represents the production of IFN-y when using the wild-type polypeptide.

Table 1 indicates that the present polypeptide acted on KG-1, an immunocompetent cell, to induce the production of IFN-γ. The IFN-γ production was equal to or higher than that induced by the wild-type polypeptide.

Example A-1(h)

Enhancement of cytotoxicity of NK cells

A fresh blood was collected from a healthy donor by using a syringe containing heparin, and the blood was diluted with the equal volume of PBS. The diluted blood was overlaid on FICOLL and centrifuged to obtain high-density lymphocytes. The lymphocytes were suspended to give a density of 1 \times 10⁶ cells/ml in RPMI-1640 medium (pH 7.2) containing 10 µg/ml kanamycin, 5 \times 10⁻⁵ M 2-mercaptoethanol and 10 v/v % fetal bovine serum. 0.5 ml of the cell suspension was put into each well of 12-well microplates. To each well, the present polypeptide or the wild-type polypeptide, obtained in Example A-1(c), in 1.5 ml solution was added after appropriately diluted with a fresh preparation of the medium, and 0.5 ml of a fresh preparation of the medium with or without 50 units/ml of a recombinant human interleukin 2 was further added. Thereafter, the cells were cultured at 37°C for 24 hours in a 5 v/v % CO₂ incubator. The cultured cells were washed with PBS to obtain cultured lymphocytes containing NK cells as effector cells.

K-562 cells, ATCC CCL243, an established cell line derived from human chronic myelogenous leukemia, as target cells sensitive to NK cells, were labelled with 51 Cr by a conventional method, and 1×10^4 cells of the labelled cells were put into each well of 96-well microplates. To the wells, the cultured lymphocytes obtained above were added to give the ratios of 2.5:1, 5:1 and 10:1 between the effector and the target cells, before cultured at 37°C for 4 hours in a 5 v/ v % CO_2 incubator. Thereafter, the culture supernatants were examined on the radioactivity by conventional manner to estimate the number of killed cells. Percentage (%) of the killed cells to the target cells tested in each system was calculated to evaluate the cytotoxicity. Table 2 shows the results.

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5		&**	[Target Cells]	9)	73 (73) 65 (66)	(2	9)	(7	(7	œ)	8)	6)	represents
10		icity,	: [Ta	\ \`	(48)	_	<u> </u>	<u> </u>	<u> </u>	_	_)	íl .
15		Cytotoxicity	Cells]) 48 3) 41	_	_	_	<u> </u>	<u> </u>	_	_	parentheses eptide.
20		0	[Effector 2.5:1	7	30 (30 25 (23) 1	8	9) () 7	<u>ო</u>	7	ie in polyp
25	Table 2		unit/ml										. **: Valu
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35		Concentration	Interleukin	•	-	-	•	-	•	7		1	a molarity oited when
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45	•	pocentration	Polypeptide, pM*	00	0.5	0.5	ın u	ນ ເ	0 0	000	000	200	NOTE) *: "pM" means a molarity the cytotoxicity exhibited when
50		Ü	, ğ										NOTE)

As shown in Table 2, the present polypeptide enhanced the cytotoxicity of NK cells, and the enhancement was equal to or higher than that of the wild-type polypeptide. The enhancement was augmented by the co-existing of interleukin 2.

Example A-1(i)

Induction of LAK cell formation

Cultured lymphocytes containing LAK cells as effector cells were prepared by a procedure similar as in Example A-1(g) excepting the culturing time replaced with 72 hours. Raji cells, ATCC CCL86, an established cell line derived from human Burkitt lymphoma, as target cells non-sensitive to NK cells, was labelled with ⁵¹Cr according to the conventional method. 1 × 10⁴ of the labelled cells were put into each well of 96-well microplates, and the cultured lymphocytes were added to the wells to give the ratios of 5:1, 10:1, and 20:1 between the effector and the target cells, before cultured at 37°C for 4 hours in a 5 v/v % CO₂ incubator. Thereafter, similarly as in Example A-1(g), the culture supernatants were examined on the radioactivity to evaluate the cytotoxicity. Table 3 shows the results.

5 10 15		Cytotoxicity, ***	[Effector Cells] : [Target Cells] 5:1 10:1 20:1	1 (11) 21 (21) 34 (15 (15) 28 (28) 38 (38) 14 (13) 24 (22) 34 (35)	8 (17) 32 (31) 42 (6 (15) 26 (23) 37 (1 (19) 36 (34) 50 (7 (23) 15 (07) 78 (83) 64 (93) 65 (83) 65 (83)	7 (27) 44 (34) 61 (3 (31) 59 (54) 72 (Value in parentheses represents type polypeptide.
25	Table 3		unit/ml									<pre>12 M. **: Valu the wild-type</pre>
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35			Interleukin 12	•	-1	-	•		•			exhibited when
40		100 OF	e, pM*									" means a ity exhib
50		Concentration	Polypeptide,	00	0.5	ດ . ທີ່	ا ما	3 C	20	200	200	NOTE) *: "pM" methe cytotoxicity

As shown in Table 3, the present polypeptide induced the formation of LAK cells, and the induction was equal to or higher than that of the wild-type polypeptide. The induction was augmented by the co-existing of interleukin 2.

Example A-1(i)

Acute toxicity test

The present polypeptide in Example A-1(c) was percutaneously, perorally or intraperitoneally administered to 8-week-old mice in usual manner. As a result, the LD₅₀ of the present polypeptide proved to be about one mg or higher per one kg of the body weight, independently of the administration routs. This evidences that the present polypeptide can be incorporated into pharmaceuticals for humans without anxiety.

Example A-2

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Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT21" containing the nucleotide sequence of SEQ ID NO:16 was obtained by a procedure similar as in Example A-1(a) but using the DNA fragment 6, obtained in Example A-1(a), as a template, and an oligonucleotide with the nucleotide sequence of 5'-CTGATTCTGACTCTAGATAATGC-3' and an oligonucleotide with the nucleotide sequence of 5'-GCATTATCTCTAGAGTCAGAATCAG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 38th position in SEQ ID NO:4 with a serine. As shown in FIG. 4, in the recombinant DNA "pCSHIGIF/MUT21", a cDNA "IGIF/MUT21" encoding the amino acid sequence of SEQ ID NO:7 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1 (b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:7 in an amount of about 50 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1 (f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-3

Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT25" containing the nucleotide sequence of SEQ ID NO:17 was obtained by a procedure similar as in Example A-1(a) but using the DNA fragment 6, obtained in Example A-1(a), as a template, and an oligonucleotide with the nucleotide sequence of 5'-CTTTCTAGCTTCTGAAAAAGAGAGAGAG-3' and an oligonucleotide with the nucleotide sequence of 5'-CTCTCTTTTTCAGAAGCTAGAAAG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 127th position in SEQ ID NO:4 with a serine. As shown in FIG. 5, in the recombinant DNA "pCSHIGIF/MUT25", a cDNA "IGIF/MUT25" encoding the amino acid sequence of SEQ ID NO:8 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype a2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:4 in an amount of about 30 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-4

Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT32" containing the nucleotide sequence of SEQ

ID NO:18 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT21" as a template, encoding the amino acid sequence of SEQ ID NO:7, in the recombinant DNA "pCSHIGIF/MUT21" obtained in Example A-2, and an oligonucleotide with the nucleotide sequence of 5'-CTTTCTAGCTTCTGAAAAAGAGAGAGAG-3' and an oligonucleotide with the nucleotide sequence of 5'-CTCTCTTTTTCAGAAGCTAGAAAG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 127th position in SEQ ID NO:4 with a serine. As shown in FIG. 6, in the recombinant DNA "pCSHIGIF/MUT32", a cDNA "IGIF/MUT32" encoding the amino acid sequence of SEQ ID NO:9 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype a2b of human interferon-a.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:9 in an amount of about 80 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-5

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Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT41" containing the nucleotide sequence of SEQ ID NO:19 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT32" as a template, with the nucleotide sequence of SEQ ID NO:18, in the recombinant DNA "pCSHIGIF/MUT32" obtained in Example A-4, and an oligonucleotide with the nucleotide sequence of 5'-CAACTCTCTCTGAGAACAA-3' and an oligonucleotide with the nucleotide sequence of 5'-TTGTTCTCAGAGGAGAGTTG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 76th position in SEQ ID NO:4 with a serine. As shown in FIG. 7, in the recombinant DNA "pCSHIGIF/MUT41", a cDNA "IGIF/MUT41" encoding the amino acid sequence of SEQ ID NO:10 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype a2b of human interferon-a.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:10 in an amount of about 6 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-6

Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT35" containing the nucleotide sequence of SEQ ID NO:20 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT21" as a template, encoding the amino acid sequence of SEQ ID NO:7, in the recombinant DNA "pCSHIGIF/MUT21" obtained in Example A-2, and an oligonucleotide with the nucleotide sequence of 5'-CTCTCCGCTGAGAACAAAATTATTTCC-3' and an oligonucleotide with the nucleotide sequence of 5'-TTTGTTCTCAGCGGAGAGAGTTG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 76th position in SEQ ID NO:4 with an alanine. As shown in FIG. 8, in the recombinant DNA "pCSHIGIF/MUT41", a cDNA "IGIF/MUT35" encoding the amino acid sequence of SEQ ID NO:11 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:11 in an amount of about 60 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in

the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-7

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Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT42" containing the nucleotide sequence of SEQ ID NO:21 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT32" as a template, encoding the amino acid sequence of SEQ ID NO:18, in the recombinant DNA "pCSHIGIF/MUT32" obtained in Example A-4, and an oligonucleotide with the nucleotide sequence of 5'-CTCTCGCTGAGAACAAAATTATTTCC-3' and an oligonucleotide with the nucleotide sequence of 5'-TTTGTTCTCAGCGGAGAGAGTTG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 76th position in SEQ ID NO:4 with an alanine. As shown in FIG. 9, in the recombinant DNA "pCSHIGIF/MUT42", a cDNA "IGIF/MUT42" encoding the amino acid sequence of SEQ ID NO:12 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:12 in an amount of about 30 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-8

Production of polypeptide

A PCR was performed similarly as the PCR for obtaining the DNA fragment 1 in Example A-1(a) but using an oligonucleotide with the nucleotide sequence of 5'-CGGCCAAAGTTGCCCACAGAGCAGCTTG-3', chemically synthesized, for the antisense primer 1. The PCR resulted in obtaining a DNA fragment (the DNA fragment 7) which comprised the nucleotide sequence of SEQ ID NO:24, a site recognized by a restriction enzyme *Xho*I, linked to the 5'-terminus of the SEQ ID NO:24, and a sequence of Ist-Ilth nucleotides in the nucleotide sequence of SEQ ID NO:28, linked to the 3'-terminus of the SEQ ID NO:24.

The recombinant DNA "pMGTG-1", containing the nucleotide sequence of SEQ ID NO:28 encoding the wild-type polypeptide with the amino acid sequence of SEQ ID NO:5, was prepared according to the methods described in Japanese Patent Kokai No.27,189/96 by the present applicant. The wild type polypeptide, with the amino acid sequence of SEQ ID NO:5, contains partial amino acid sequences of SEQ ID NOs:1, 2 and 3 in the parts consisting of 16th-21st, 29th-34th, and 50th-54th amino acids, respectively. Oligonucleotides with the nucleotide sequence of 5'-CTGCTCT-GTGGGCAACTTTGGCCGACTTCACTG-3' as a sense primer (the sense primer 3) and 5 '-ACACGCG-GCCGCCTAACTTTGATGTAAGTTAG-3' as an antisense primer (the antisense primer 3) were chemically synthesized. Thereafter, a PCR was performed similarly as that for obtaining the DNA fragment 2 in Example A-1 (a) but using the recombinant DNA "pMGTG-1", the sense primer 3 and the antisense primer 3 for the recombinant DNA "pHIGIF", the sense primer 2 and the antisense primer 2, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 8) which comprised the nucleotide sequence of SEQ ID NO:28, a termination codon of 5'-TAG-3' and a site recognized by a restriction enzyme Nofi, linked to the 3'-terminus of the SEQ ID NO:28, and a sequence of 57th-69th nucleotides in the nucleotide sequence of SEQ ID NO:24, linked to the 5'-terminus of the SEQ ID NO:28.

A PCR was performed similarly as that for obtaining the DNA fragment 3 in Example A-1(a) but using the DNA fragments 7 and 8 and the antisense primer 3, obtained above, for the DNA fragments 1 and 2 and the antisense primer 2, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 9) comprising the nucleotide sequence of SEQ ID NO:29.

A PCR was performed similarly as that for obtaining the DNA fragment 4 in Example A-1(a) but using the DNA fragment 9 for, the DNA fragment 3, the antisense primer 3 for the antisense primer 2, and an oligonucleotide with the

nucleotide sequence of 5'-GGCCGACTTCACGCTACAACC-3' for the mutagenic sense primer, to replace 103rd and 104th nucleotides of thymine and guanine in SEQID NO:29 with a guanine and cytosine, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 10) comprising a nucleotide sequence identical to 91st-570th nucleotides in SEQID NO:29 except for the 103rd and 104th replaced with a guanine and a cytosine, respectively.

A PCR was performed similarly as that for obtaining the DNA fragment 5 in Example A-1(a) but using the DNA fragment 9 for the DNA fragment 3, and an oligonucleotide with the nucleotide sequence of 5'-GGTTGTAGCGT-GAAGTCGGCC-3' for the mutagenic antisense primer, to replace 103rd and 104th nucleotides of thymine and guanine in SEQ ID NO:29 with a guanine and cytosine, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 11) comprising a nucleotide sequence identical to 1st-111th nucleotides in SEQ ID NO:29 except for the 103rd and 104th, replaced with a guanine and cytosine, respectively.

A PCR was performed similarly as that for obtaining the DNA fragment 3 in Example A-1(a) but using the DNA fragments 10 and 11 and the antisense primer 3, obtained above, for the DNA fragments 1 and 2 and the antisense primer, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 12) comprising the nucleotide sequence of SEQ ID NO:22, the nucleotide sequence of SEQ ID NO:24 and a site recognized by a restriction enzyme Xhol, linked to the 5'-terminus of the SEQ ID NO:22, and a termination codon of 5'-TAG-3' and a site recognized by a restriction enzyme Noti, linked to the 3'-terminus of the SEQ ID NO:22.

The DNA fragment 12 was treated similarly as the DNA fragment 6, according the procedure for obtaining the recombinant DNA "pCSHIGIF/MUT12" in Example A-1(a), to obtain a autonomously replicable recombinant DNA "pCSMIGIF/MUT11". As shown in FIG. 10, in the recombinant DNA "pCSMIGIF/MUT11", a cDNA "mIGIF/MUT11" with the nucleotide sequence of SEQ ID NO:22 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α. As shown in the accompanied amino acid sequence, the SEQ ID NO:22 encodes an amino acid sequence derived from the wild type polypeptide with SEQ ID NO:5 by replacing the cysteine at the 7th position with an alanine.

For a control, an autonomously replicable recombinant DNA "pCSMIGIF/WT" was prepared similarly as the procedure for obtaining the recombinant DNA "pCSHIGIF/MUT12" but treating the DNA fragment 9 for the DNA fragment 6. As shown in FIG. 11, in the recombinant DNA "pCSMIGIF/WT", a cDNA "mIGIF/WT" with the nucleotide sequence of SEQ ID NO:28, encoding the wild-type polypeptide, was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of subtype a2b of human interferon-α.

Example A-8(b)

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Production of polypeptide by transformant

According to the procedure for the production of the polypeptide in Example A-1(b) but using the recombinant DNA "pCSMIGIF/MUT12" for "pCSHIGIF/MUT11", the recombinant DNA was extracted, the DNA was introduced into COS-1 cells, and the COS-1 cells with the DNA was cultured to obtain a culture. The culture was analyzed by Western blotting using the monoclonal antibody described in Japanese Patent Kokai No.217,798/96 by the present applicant. The analysis proved that the present polypeptide capable of inducing production of IFN-γ by immunocompetent cells, with the amino acid sequence derived from SEQ ID NO:5 by replacing the cysteine at 7th position with an alanine, was produced in the culture in an amount of about 20 ng/ml.

As a control, treating the recombinant DNA "pCSHMIGIF/WT" similarly as above produced the wild-type polypeptide capable of inducing production of IFN-γ by immunocompetent cells. The production of the wild-type polypeptide was significantly lower than that obtained by using "pCSMIGIF/MUT11", descrived above. This evidences that the present polypeptide in this Example is more stable and exhibits the biological activities higher than the wild-type polypeptide.

Example A-8(c)

Purification of polypeptide

The culture containing the present polypeptide, in Example A-8(b), was centrifuged to collect a supernatant. The supernatant was fed to a column packed with a gel for immunoaffinity chromatography using the monoclonal antibody, prepared by the method described in Japanese Patent Kokai No.217,798/96 by the present applicant, and preliminarily washed with PBS. After a fresh PBS was run through the column to wash, 35 mM ethylamine (pH 10.8) was run to elute. From the eluted fractions, those containing the polypeptide capable of inducing production IFN- γ by immunocompetent cells were collected. The collected fractions were dialyzed against PBS at 4°C for 18 hours, and then concentrated by membrane-filtration followed by lyophilization to obtain a solid polypeptide with a purity of about 95%. In parallel, the culture containing the wild-type polypeptide, obtained by using the recombinant DNA *pCSMIGIF/

WT", was purified similarly as above for a control in analyzing the physicochemical properties as described below.

Example A-8(d)

5 Molecular weight

SDS-Polyacrylamide gel electrophoresis of the present polypeptide in Example A-8(c), similarly as in Example A-1(d), exhibited a main band of polypeptide capable of inducing production at a position corresponding to a molecular weight of about 18,500-19,500 daltons.

Example A-8(e)

N-Terminal amino acid sequence

By analyzing similarly as in Example A-1(e), the present polypeptide in Example A-8(c) was proved to contain the amino acid sequence of SEQ ID NO:30 in the N-terminus.

Example A-8(f)

20 Stability

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The present polypeptide or the wild-type polypeptide, in Example A-8(c), was dissolved in PBS containing 0.2 g/ml maltose, and the solution was incubated at 40°C for 24 hours. After 0, 3, 9, or 24 hours from starting the incubation, a portion of each solution was sampled. The samples were individually assayed on IFN-γ inducing activity, according to the methods described below, in Example A-8(g), to study the time course of the activity upon the incubation. Percentage (%) of the residual activity at every point was calculated based on the activity at the starting point. The results are in FIG. 12.

As shown in FIG. 12, the present polypeptide in this Example was more stable and retained the activity longer than the wild-type polypeptide. This evidences that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example A-8(g)

Production of IFN-γ by immunocompetent cells

Splenocytes were collected from C3H/HeJ mice as immunocompetent cells. The splenocytes were suspended in RPMI-1640 medium supplemented with 10 v/v % fetal bovine serum. The suspensions were given the present polypeptide or the wild-type polypeptide, in Example A-8(a), in the presence or absence of concanavalin A or interleukin 2. Thereafter, the splenocytes were cultured before examined on productions of IFN- γ by conventional enzyme-immunoassay to evaluate an inducing activity of production of IFN- γ . The present polypeptide proved to act on the splenocytes, immunocompetent cells, to induce the production of IFN- γ . The inducing activity of IFN- γ production of the present polypeptide was equal to or higher than that of the wild-type polypeptide.

Example A-8(h)

Acute toxicity test

The present polypeptide in Example A-8(a) was examined on the acute toxicity by the method in Example A-1(j). As a result, the LD₅₀ of the present polypeptide proved to be about one mg or higher per one kg of the body weight, independently of the administration routs. This evidences that the present polypeptide can be incorporated into pharmaceuticals for mammalian including humans without anxiety.

Example A-9

55 Production of polypeptide

An autonomously replicable recombinant DNA "pCSMIGIF/MUT12" containing the nucleotide sequence of SEQ ID NO:23 was obtained by a procedure similar as in Example A-8(a) but using the DNA fragment 9, obtained in Example

A-8(a), as a template, and an oligonucleotide with the nucleotide sequence of 5'-GGACACTTTCTT-GCTAGCCAAAAGG-3' and an oligonucleotide with the nucleotide sequence of 5'-CCTTTTGGCTAGCAAGAAAGT-GTCC-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 125th position in SEQ ID NO:5 with a serine. As shown in FIG. 13, in the recombinant DNA "pCSMIGIF/MUT12", a cDNA "mIGIF/MUT12" encoding the amino acid sequence of SEQ ID NO:14 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:14 in an amount of about 50 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-8. As a result, the polypeptide in this Example proved to be similar to that in Example A-8 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 12, the results of the analysis on stability, obtained according to the method in Example A-8(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

Example B-1

Solution

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Any one of the present polypeptides purified in Examples A-1 to A-9 was dissolved in physiological saline containing one v/v % human serum albumin as a stabilizer to give a concentration of one mg/ml, and the solution was membrane-filtered in usual manner into a germ-free solution.

The solutions, with a satisfactory stability, can be used as an injection, an ophthalmic solution, and a collunarium for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections and immunopathies, of mammalian including human.

Example B-2

30 Dry injection

One hundred mg of any one of the present polypeptides purified in Examples A-1 to A-9 was dissolved in 100 ml of physiological saline containing one w/v % gelatin as a stabilizer, and the solution was sterilized membrane-filtered in usual manner into a germ-free solution. One ml aliquotes of each of the sterilized solutions were distributed to vials, and lyophilized before sealing the vials with caps.

The products, with a satisfactory stability, can be used as a dry injection for treating and/or preventing susceptive diseases such as matignant tumors, viral diseases, infections, and immunopathies of mammalian including human.

Example B-3

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Ointment

"HI-BIS-WAKO 104", a carboxyvinylpolymer commercialized by Wako Pure Chemicals, Tokyo, Japan, and "TRE-HALOSE", a powdered crystalline trehalose commercialized by Hayashibara Co., Ltd., Okayama, Japan, were dissolved in sterilized distilled water to give concentrations of 1.4 w/w % and 2.0 w/w %, respectively. Any one of the present polypeptides purified in Examples A-1 to A-9 was mixed with the solution into homogeneity. Each of the homogenate was adjusted to pH 7.2 to obtain a paste containing about one mg/g of any one of the polypeptides.

The pastes, with a satisfactory spreadablity and stability, can be used as an ointment for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections and immunopathies, of mammalian including human.

Example B-4

Tablet

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Any one of the purified polypeptides in Examples A-1 to A-9 and "LUMIN", [bis-4-(1-ethylquinoline)][γ -4'-(1-ethylquinoline)] pentamethionine cyanine, as a cell activator, were mixed with "FINETOSE®, an anhydrous crystalline α -maltose commercialized by Hayashibara Co., Ltd., Okayama, Japan, into homogeneity. Each of the homogeneite was

processed with a conventional tablet machine into tablets, each of which weighed 200 mg and contained about one mg of any of the polypeptides and the LUMIN.

The tablets with a satisfactory swallowability, stability and cell-activating activity can be used as a tablet for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections and immunopathies, of mammalian including human.

Example B-5

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Adoptive immunotherapeutic agent

Mononuclear cells were isolated from a peripheral blood of a patient with malignant lymphoma. The cells were suspended in RPMI-1640 medium supplemented with 10 v/v % human AB serum, preheated at 37°C, to give a density of 1×10⁶ cells/ml. To the cell suspension, any one of the present polypeptides in Examples A-1 to A-7 and a recombinant human interleukin 2 were added as adoptive immunotherapeutic agent to give concentrations of 10 ng/ml and 100 units/ml, respectively, before the cells were cultured at 37°C for one week in a 5 v/v % CO₂ incubator. Thereafter, the culture was centrifuged to collect LAK cells.

The LAK cells can exhibit so strong cytotoxicity to the lymphoma when returned to the patient, and an adoptive immunotherapy using the present agent can exert significantly higher effect than that using the interleukin 2 alone. Cytotoxic T cells obtained similarly as above excepting the mononuclear cells, replaced with tumor-invasive lymphocytes, also can effect as equivalent to that of the LAK cells, when returned to the patient. Thus the adoptive immunotherapeutic agent in this Example can be effectively applied to solid malignant tumors such as renal cancer, malignant melanoma, colonic cancer, rectal cancer, and lung cancer, besides malignant lymphomas.

IFN-γ is well known to be involved in protection against infections of virus and bacteria, etc., inhibition of malignant tumors proliferation, regulation of immune system causing protection, and inhibition of immunoglobulin E antibodies production. And IFN-γ is now in use for agents against human susceptive diseases, stating that the directions for the targeting diseases, uses, dosages, and safeness have been already established.

As described in a publication as Frances R. Balkwill, Saitokain-To-Ganchiryo (Cytokines in Cancer Therapy), Yoshi-hiko WATANABE tr., (Tokyo, Japan: Tokoyo Kagaku Dojin Co., Ltd., 1991), therapies using killer cells such as NK cells and LAK cells that include antitumor immunotherapies are applied to human diseases, resulting in satisfactory effects as a whole. Recently, an intensive interest is taken in the involvement of the killer cells, which have cytotoxicities enhanced by cytokines, or which are formed induced by cytokines, in therapeutic effects. For example, T. Fujioka et al., British Journal of Urology, Vol.73, No.1, pp.23-31 (1994) describes that in an antitumor immunotherapy using both LAK cells and interleukin 2, the interleukin 2 induced formation of the LAK cells, resulting in remarkable effects against human cancer metastases without exhibiting serious toxicities and side effects.

Thus, it has been revealed that IFN-γ or killer cells are involved in treatment and/or prevention of a variety of human diseases, and can contribute to cure or remission to the diseases. As shown in Examples A-1 to A-9, the present polypeptides induce the production of IFN-γ by immunocompetent cells, enhance the cytotoxicity of NK cells, and induce the formation of LAK cells, indicating that the present agents for susceptive diseases can be administered to patients successively for a relatively-long period of time, and effect to treat and/or prevent diseases, in which IFN-γ and/or killer cells are involved, without causing serious side effects.

[Effect of the invention]

As described above, the present invention is made based on the establishment of stable polypeptides capable of inducing production of IFN- γ by immunocompetent cells. The polypeptides according to the present invention are the substances clarified on their amino acid sequence, and feature to retain the biological activities for a relatively-long period in actual use, because of the higher stability than that of the wild-type polypeptide. Thus the present polypeptides provide a variety of uses such as an IFN- γ inducer for producing IFN- γ in cell cultures and an agent for treating and/or preventing diseases sensitive to IFN- γ in general, including viral diseases, infections, malignant tumors, and immunopathies. The agents with the present polypeptides additionally possessing properties of enhancing cytotoxicities and/or inducing formation of killer cells, as effective ingredients, can satisfactorily treat serious diseases such as malignant tumors.

Furthermore, the present polypeptides generally can induce a desired level of IFN- γ with only a slight amount since they have so strong activity of inducing production of IFN- γ . Because of little toxicity, the polypeptides wouldn't cause serious side effects even when administered with relatively-high doses. These give the present polypeptides an advantage of that they can induce a desired level of IFN- γ rapidly without strictly controll on the dosages in actual use. The polypeptides with these usefulness can be easily produced in a desired amount by the present process using recombinant DNA techniques.

The present invention is a significant invention which has a remarkable effect and gives a great contribution to this field.

While there has been described what is at present considered to be the preferred embodiments of the present invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

SEQUENCE LISTING

	(1) GENA	RAL INFORMATION:
5	(i)	APPLICANT: NAME:KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO
	(ii)	TITLE OF INVENTION: POLYPEPTIDES
10	(iii)	NUMBER OF SEQUENCES:30
15	(iv)	ADDRESS: (A) ADDRESSEE: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO (B) STREET: 2-3, 1-CHOME, SHIMOISHII (C) CITY: OKAYAMA (E) COUNTRY: JAPAN (F) POSTAL CODE (ZIP): 700
20	(v)	COMPUTER READABLE FORM: (A) MEDIUM TYPE:Floppy disk (B) COMPUTER:IBM compatible (C) OPERATING SYSTEM:PC-DOS/MS-DOS
25	(vii)	PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: JP 333,037/96 (B) FILING DATE: November 29, 1996 (A) APPLICATION NUMBER: JP 20,906/97 (B) FILING DATE: January 21, 1997
30		(A) APPLICATION REFERENCE NO: JP 10,053,503 (B) FILING DATE: November 14, 1997
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              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
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        Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
45
                    20
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                35
                                     40
        Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                                 55
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
50
                             70
                                                 75
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                         85
                                              90
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                                         105
55
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                115
                                     120
                                                          125
```

```
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                  135
                                                       140
         Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
         145
5
         (11) INFORMATION FOR SEQ ID NO:10:
               (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 157 amino acids
                    (B) TYPE: amino acid
10
                    (D) TOPOLOGY: linear
               (ii)MOLECULE TYPE:peptide
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
         Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
15
         Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                     20
                                          25
         Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                                      40
         Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
20
         Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile
                              70
         Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                          85
                                              90
         Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                     100
                                          105
         Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                      120
                                                           125
         Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                  135
                                                       140
30
         Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
         145
                              150
         (12) INFORMATION FOR SEQ ID NO:11:
35
               (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
40
         Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
         Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                                          25
45
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                                      40
         Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                                  55
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
                             70
                                                  75
50
         Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                          85
                                               90
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                     100
                                          105
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
55
                                      120
        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
```

135

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Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
                              150
5
         (13) INFORMATION FOR SEQ ID NO:12:
               (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
10
               (ii) MOLECULE TYPE: peptide
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
         Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
15
         Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                                      40
         Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
20
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
                             70
         Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                                              90
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
25
                     100
                                          105
                                                               110
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                      120
        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                 135
        Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
30
        145
         (14) INFORMATION FOR SEQ ID NO:13:
               (i) SEQUENCE CHARACTERISTICS:
35
                   (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
40
        Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn
        Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
        Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
45
        Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
                                 55
        Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile
                             70
        Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
50
        Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
                                          105
        Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
                                     120
        Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
                                 135
                                                      140
```

Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser

150

5 (15) INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 157 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14: Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn 10 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 15 20 25 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile 40 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 55 60 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 20 70 75 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 100 105 25 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Ser Gln Lys Glu 120 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 130 135 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 150 30 (16) INFORMATION FOR SEQ ID NO:15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 471 base pairs 35 (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE:cDNA (iX) FEATURE: (A) NAME/KEY: mat peptide 40 (B) LOCATION: 1..471 (C) IDENTIFICATION METHODS:S (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15: TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT 4 R 45 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 GAC CAA GTT CTC TTC ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT 96 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 ATG ACT GAT TCT GAC TGT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT 144 50 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 35 40 45 ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 60 TCT GTG AAG TCT GAG AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT 55 240 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile

	65 ATT	TCC	TTT	AAG	GAA	70 ATG	AAT	ССТ	ССТ	GAT	75 AAC	ATC	مدد	CAT	N C N	80	200
5	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr	Lys	288
	AGT Ser	GAC Asp	ATC Ile	ATA Ile 100	TTC Phe	TTT Phe	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT Asn	AAG Lys	336
10	ATG Met	CAA Gln	TTT Phe 115	GAA Glu	TCT Ser	TCA Ser	TCA Ser	TAC Tyr 120	GAA	GGA Gly	TAC Tyr	TTT Phe	Leu	GCT	TGT Cys	GAA Glu	384
	AAA Lys	Glu	AGA Arg	GAC Asp	CTT Leu	TTT Phe	Lys	CTC	ATT Ile	TTG Leu	AAA Lys	Lys	125 GAG Glu	GAT Asp	GAA Glu	TTG Leu	432
15	GGG Gly 145	130 GAT Asp	AGA Arg	TCT Ser	ATA Ile	ATG Met 150	135 TTC Phe	ACT Thr	GTT Val	CAA Gln	AAC Asn 155	140 GAA Glu	GAC Asp				471
20	(17			QUEN(A) LEI	CE CI NGTH		TER:	ISTIC e pa:	CS:								
		_	((C) STE D) TOE DLECT	RANDI POLOC JLE 1	EDNES SY:li	SS:do .nea:	ouble c	Э								
25		(2	(I	3) LOC	CATIO	EY:ma ON:1. FICAT DESCR	.471 ION	L METH	HODS):16:	:					
30	Tyr 1	Phe	GGC Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn	48
	GAC Asp	Gln	GTT Val	CTC leu 20	TTC Phe	Ile	GAC Asp	CAA Gln	GGA Gly 25	AAT Asn	CGG	CCT Pro	CTA Leu	TTT Phe 30	GAA Glu	GAT Asp	96
35	ATG Met	ACT Thr	GAT Asp 35	TCT Ser	GAC Asp	TCT Ser	AGA Arg	GAT Asp 40	AAT Asn	GCA Ala	CCC Pro	CGG Arg	ACC Thr 45	ATA Ile	TTT Phe	ATT Ile	144
	Ile	Ser 50	ATG Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	GCT Ala	Val	Thr	Ile	192
40	Ser 65	Val	AAG Lys	Ser	Glu	Lys 70	ATT Ile	Ser	Thr	Leu	Ser 75	TGT Cys	Glu	Asn	Lys	Ile 80	240
45	Ile	Ser	TTT Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	288
	AGT Ser	GAC Asp	ATC Ile	ATA Ile 100	TTC Phe	TTT Phe	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT Asn	AAG Lys	336
50	Met	Gln	TTT Phe 115	Glu	Ser	Ser	Ser	Tyr 120	GAA Glu	Gly	Tyr	Phe	Leu 125	GCT Ala	Cys	Glu	384
	AAA Lys	GAG Glu 130	AGA Arg	GAC Asp	CTT Leu	TTT Phe	AAA Lys 135	CTC Leu	ATT Ile	TTG Leu	AAA Lys	AAA Lys 140	GAG	GAT Asp	GAA Glu	TTG Leu	432
<i>55</i>	GGG Gly 145	GAT Asp	AGA Arg	TCT Ser	ATA Ile	ATG Met	TTC	ACT Thr	GTT Val	CAA Gln	AAC Asn	GAA	GAC Asp				471

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(18) INFORMATION FOR SEQ ID NO:17:
            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 471 base pairs
                (B) TYPE: nucleic acid
5
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
           (ii) MOLECULE TYPE:cDNA
            (ix) FEATURE:
                (A) NAME/KEY: mat peptide
10
                (B) LOCATION: 1..471
                (C) IDENTIFICATION METHODS:S
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
     TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
                                                                            48
     Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
                                           10
     GAC CAA GTT CTC TTC ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT
     Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                 20
                                      25
     ATG ACT GAT TCT GAC TGT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT
                                                                           144
20
     Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                                  40
                                                       45
     ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC
                                                                           192
     Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
         50
                              55
                                                   60
     TCT GTG AAG TCT GAG AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT
25
                                                                           240
     Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
                          70
                                               75
     ATT TCC TTT AAG GAA ATG AAT CCT CCT GAT AAC ATC AAG GAT ACA AAA
                                                                           288
     Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                                           90
     AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAT AAG
30
                                                                           336
    Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                 100
                                      105
    ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TCT GAA
                                                                           384
    Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                  120
                                                       125
35
    AAA GAG AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG
                                                                           432
    Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                              135
    GGG GAT AGA TCT ATA ATG TTC ACT GTT CAA AAC GAA GAC
                                                                           471
    Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
     145
40
     (19) INFORMATION FOR SEQ ID NO:18:
           (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 471 base pairs
45
               (B) TYPE: nucleic acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear
           (ii) MOLECULE TYPE:cDNA
           (iX) FEATURE:
               (A) NAME/KEY: mat peptide
50
               (B) LOCATION: 1..471
               (C) IDENTIFICATION METHODS:S
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
    TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
55
    Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
```

	1				5					10			•		15		
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
	Asp	GIn	Val		Phe	Ile	Asp	Gln		Asn	Arg	Pro	Leu		Glu	Asp	
5	ΔTG	ΔСТ	САТ	20 TCT	GAC	TOT	AGA	СУТ	25 מאת	CCN	ccc	ccc	7.00	30		.	
	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Ara	Thr	AIA	Pho	ATT	144
			35					40				_	45				
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly		Ala	Val	Thr	Ile	
10	тст	50 CTC	7 7 C	T CT	CAC		55	TCA	» om	cmc	maa	60 TO					
	Ser	Val	LVC	Sar	GAG	AAA	ATT Ile	Cor	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240
	65	• • • •	פעם	261	GIU	70	116	SCI	1111	neu	75	Cys	GIU	ASI	гàг		
		TCC	TTT	AAG	GAA		AAT	CCT	CCT	GAT		ATC	AAG	GAT	ACA	80 444	288
15	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lvs	200
,3					85					90				_	95	-	
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	ser	Asp	TTE		Pne	Pne	Gln	Arg	Ser	Val	Pro	Gly	His	_	Asn	Lys	
	ATG	CAA	ተጥተጥ	100 GAA	тст	ጥሮል	TCA	ጥአሮ	105	CGA	ጥአ ሮ	שהטונט	CTA	110	mom	~~~	
20	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tvr	Glu	Glv	TVY	Phe	T.e.11	Δla	Cor	GAA	384
			115					120					125				
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
	ccc	130	202	m.c.m	2002	.	135	. cm		~~~		140					
25	GGG	DED	AGA	Sor	TIA	Mot	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
	145	тар	Arg	261		150	Phe	1111	vai	GIII	155	GIU	Asp				
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	(20)	TNI	r∩DM1	ነጥፐ () ነ	I EOI	0 000	Q ID	NO.	٠.								
30	(20)						TERI										
-							base										
			(E	3) TY I	E:ni	ıclei	c ac	id									
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35			X) FE			IPE:	CDNA	.									
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		()	(i)SE	EQUE	ICE I	ESCF	RIPTI	ON: 5	SEQ 1	D NC	:19:	:					
40	TAC	ተተ	GGC	AAC	ست	CAA	TCT	* * *	מיחים	m C a	ama	2002	202				
	Tvr	Phe	Glv	Lvs	Leu	Glu	Ser	LVS	Len	Ser	Val	ATA	AGA	AAT	TIG	AAT	48
	1		1	-1-	5		001	Dy 3	Deu	10	vai	TTC	ALG	ASII	15	ASII	
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
45	Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
45	3 000	» cm	a s m	20	~~~	mam			25					30			
	Mot	Thr	ACD	Cor	ACD	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
		T 11 T	ASU	Ser	ASD	ser	Arg		ASII	Ala	Pro	Arg	1nr 45	TIE	Phe	Ile	
			35		•												
			35				AGC	40 CAG	ССТ	AGA	GGT	ATG		СΤЪ	ΔСТ	ልጥሮ	192
50	ATA	AGT	35 ATG	TAT	AAA	GAT	AGC Ser	CAG	CCT Pro	AGA Arq	GGT Gly	ATG Met	GCT	GTA Val	ACT Thr	ATC Ile	192
50	ATA Ile	AGT Ser 50	35 ATG Met	TAT Tyr	AAA Lys	GAT Asp	Ser 55	CAG Gln	Pro	Arg	Gly	Met 60	GCT Ala	Val	Thr	Ile	192
50	ATA Ile TCT	AGT Ser 50 GTG	35 ATG Met AAG	TAT Tyr TCT	AAA Lys GAG	GAT Asp AAA	Ser 55 ATT	CAG Gln TCA	Pro ACT	Arg	Gly TCC	Met 60 TCT	GCT Ala GAG	Val AAC	Thr	Ile ATT	192 240
50	ATA Ile TCT Ser	AGT Ser 50 GTG	35 ATG Met AAG	TAT Tyr TCT	AAA Lys GAG	GAT Asp AAA Lys	Ser 55	CAG Gln TCA	Pro ACT	Arg	Gly TCC Ser	Met 60 TCT	GCT Ala GAG	Val AAC	Thr	Ile ATT Ile	
50	ATA Ile TCT Ser 65	AGT Ser 50 GTG Val	35 ATG Met AAG Lys	TAT Tyr TCT Ser	AAA Lys GAG Glu	GAT Asp AAA Lys 70	Ser 55 ATT Ile	CAG Gln TCA Ser	Pro ACT Thr	Arg CTC Leu	Gly TCC Ser 75	Met 60 TCT Ser	GCT Ala GAG Glu	Val AAC Asn	Thr AAA Lys	Ile ATT Ile 80	240
50 55	ATA Ile TCT Ser 65 ATT	AGT Ser 50 GTG Val	35 ATG Met AAG Lys	TAT Tyr TCT Ser	AAA Lys GAG Glu GAA	GAT Asp AAA Lys 70 ATG	Ser 55 ATT Ile AAT	CAG Gln TCA Ser	Pro ACT Thr CCT	Arg CTC Leu GAT	Gly TCC Ser 75 AAC	Met 60 TCT Ser	GCT Ala GAG Glu AAG	Val AAC Asn GAT	Thr AAA Lys ACA	Ile ATT Ile 80 AAA	
	ATA Ile TCT Ser 65 ATT	AGT Ser 50 GTG Val	35 ATG Met AAG Lys	TAT Tyr TCT Ser	AAA Lys GAG Glu GAA	GAT Asp AAA Lys 70 ATG	Ser 55 ATT Ile	CAG Gln TCA Ser	Pro ACT Thr CCT	Arg CTC Leu GAT	Gly TCC Ser 75 AAC	Met 60 TCT Ser	GCT Ala GAG Glu AAG	Val AAC Asn GAT	Thr AAA Lys ACA	Ile ATT Ile 80 AAA	240

	Ser	GAC Asp	Ile	11e	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp	Asn	Lys	336
5	Met	Gin	115	GIu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	GCT Ala	Ser	GAA Glu	384
	Lys	GAG Glu 130	Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	GAG Glu	GAT Asp	GAA Glu	TTG Leu	432
10	GGG Gly 145	GAT Asp	AGA Arg	TCT Ser	ATA Ile	ATG Met 150	TTC Phe	ACT Thr	GTT Val	CAA Gln	AAC Asn 155	GAA Glu	GAC Asp				471
15	(21) IN	i)SE(() ()	QUEN A) LE B) TY C) ST	CE C NGTH PE:n: RAND	R SEGHARAGE : 471 ucle: EDNE: GY:1:	CTER base ic ac SS:do	ISTIC e pa cid oubl	CS: irs								
20		(:	ii)MG iX)F! () ()	OLEC' EATU A) NAI B) LO C) ID!	ULE ' RE: ME/K! CATIO ENTI!	TYPE EY:ma ON:1 FICA:	cDN at pe 471 FION	A eptic L METI	de HODS SEQ :		3 ⋅ 20						
25																	
	Tyr 1	TTT	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn	48
30	Asp	CAA Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glü	Asp	96
	Met	ACT	Asp 35	Ser	Asp	Ser	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr 45	Ile	Phe	Ile	144
35	Ile	AGT Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	192
	Ser 65	GTG Val	Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Ala	Glu	Asn	Lys	Ile 80	240
40	Ile	TCC	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr	Lys	288
	Ser	GAC Asp	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	336
. 45	Met	CAA Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu	384
50	Lys	GAG Glu 130	Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu	GAT Asp	GAA Glu	TTG Leu	432
50	GGG Gly 145	GAT Asp	AGA Arg	TCT Ser	ATA Ile	ATG Met 150	TTC Phe	ACT Thr	GTT Val	CAA Gln	AAC Asn 155	GAA Glu	GAC Asp				471

(22) INFORMATION FOR SEQ ID NO:21: (i) SEQUENCE CHARACTERISTICS:

```
(A) LENGTH: 471 base pairs
                 (B) TYPE: nucleic acid
                 (C) STRANDEDNESS: double
                 (D) TOPOLOGY: linear
5
            (ii) MOLECULE TYPE:cDNA
            (iX) FEATURE:
                 (A) NAME/KEY: mat peptide
                 (B) LOCATION: 1..471
                 (C) IDENTIFICATION METHODS:S
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
10
      TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
                                                                             48
      Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
                                            10
                                                                 15
      GAC CAA GTT CTC TTC ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT
                                                                             96
15
      Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                   20
                                        25
                                                             30
      ATG ACT GAT TCT GAC TCT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT
                                                                            144
     Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
               35
                                   40
                                                        45
      ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC
                                                                            192
20
      Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                               55
      TCT GTG AAG TCT GAG AAA ATT TCA ACT CTC TCC GCT GAG AAC AAA ATT
      Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
      65
                            70
                                                75
                                                                     80
     ATT TCC TTT AAG GAA ATG AAT CCT CCT GAT AAC ATC AAG GAT ACA AAA
                                                                           288
     Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                       85
                                            90
     AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAG
     Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                  100
                                       105
                                                            110
     ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TCT GAA
30
                                                                           384
     Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
              115
                                   120
                                                       125
     AAA GAG AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG
     Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                              135
                                                   140
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     GGG GAT AGA TCT ATA ATG TTC ACT GTT CAA AAC GAA GAC
                                                                           471
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     145
      (23) INFORMATION FOR SEQ ID NO:22:
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            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 471 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE:cDNA
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            (ix) FEATURE:
              (A) NAME/KEY: mat peptide
                (B) LOCATION: 1..471
                (C) IDENTIFICATION METHODS:S
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
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     AAC TTT GGC CGA CTT CAC GCT ACA ACC GCA GTA ATA CGG AAT ATA AAT
                                                                            48
     Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn
                                           10
     GAC CAA GTT CTC TTC GTT GAC AAA AGA CAG CCT GTG TTC GAG GAT ATG
     Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
55
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				20					25					30			
	ACT	GAT	ATT	GAT	CAA	AGT	GCC	AGT	GAA	CCC	CAG	ACC	AGA	CTG	ATA	ATA	144
	Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile	
5	ma c	N TO C	35	222	C > C	3 C M	~	40		~~`		~ ~	45				
	TVY	Met	TUY	LVC	GAC Asp	AGI	GAA	Unl	AGA	GGA	CTG	GCT	GTG	ACC	CTC	TCT	192
	-7-	50	- 7 -	2,3	nop	DCI	55	val	nrg	Gry	пеп	60	vaı	Int	Leu	ser	
	GTG	AAG	GAT	AGT	AAA	ATG	TCT	ACC	CTC	TCC	TGT	AAG	AAC	AAG	ATC	ATT	240
	Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile	
10	65 866		G > G		3.00	70					75					80	
	TCC	TTT	GAG	GAA	ATG Met	GAT	CCA	CCT	GAA	AAT	ATT	GAT	GAT	ATA	CAA	AGT	288
	561	FIIC	GIU	GIU	85	vaħ	PIO	PIO	GIU	90	116	ASP	ASP	11e	95	Ser	
	GAT	CTC	ATA	TTC	TTT	CAG	AAA	CGT	GTT		GGA	CAC	AAC	AAG	ATG	GAG	336
15	Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu	330
				100					105					110			
	TTT	GAA	TCT	TCA	CTG	TAT	GAA	GGA	CAC	TTT	CTT	GCT	TGC	CAA	AAG	GAA	384
	FIIE	GIU	115	ser	Leu	tÀt	GIU	120	HIS	Pne	Leu	Ala	125	GIn	Lys	Glu	
	GAT	GAT		TTC	AAA	CTC	ATT		AAA	AAA	AAG	GAT		AAT	GGG	GAT	432
20	Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp	132
		130					135					140			-	•	
	AAA	TCT	GTA	ATG	TTC	ACT	CTC	ACT	AAC	TTA	CAT	CAA	AGT				471
	145	Ser	val	Mec	Phe	150	Leu	IIII	ASII	Leu	155	GIN	ser				
						130					133						
25																	
	(24)				V FO												
		()			CE CH NGTH:												
					E:ni												
30			((C) STE	RANDI	EDNES	SS:do	ouble	<u> </u>								
		, ,	(r) TOI	POLOC	3Y:li	inear	-									
					** - ~												
					JLE 1	YPE:											
			X) FE	EATUI	RE:		CDNA	Ą	le								
			X) FE	IUTAS IAN (A		EY:ma	cDNA	A eptic	le								
35		(i	X) FE (<i>I</i> (E	EATUI A) NAN B) LOC C) IDI	RE: ME/KE CATIO ENTIF	EY:ma DN:1. FICAT	cDNA at pe 471 CION	A eptic L METH	iods :								
35		(i	X) FE (<i>I</i> (E	EATUI A) NAN B) LOC C) IDI	RE: ME/KE CATIO	EY:ma DN:1. FICAT	cDNA at pe 471 CION	A eptic L METH	iods :):23:						
35	AAC	i) k)	X) FE (F (E (C (i) SE	EATUR A) NAM B) LOC C) I DE EQUEN	RE: ME/KE CATIO ENTIE NCE I	EY:ma DN:1. FICAT DESCR	t pe .471 CION RIPTI	eptic METH	IODS:	D NC			CGG	ТАА	מדמ	ΔΔΤ	48
35	AAC Asn	i) k) TTT	X) FE (A (E (C (i) SE GGC	EATUI A) NAM B) LOC C) I DI EQUEN CGA	RE: ME/KE CATIO ENTIF NCE I	EY:ma ON:1. FICAT DESCE	t pe .471 CION RIPTI	PPTION:S	IODS : SEQ I	D NO	GTA	ATA	CGG Arg	AAT Asn	ATA Ile	AAT Asn	48
<i>35</i>	Asn 1	(i (x TTT Phe	(X) FE (A (E (C (C) SE (G) SE (G) SE	EATUR A) NAM B) LOC C) IDR EQUEN CGA Arg	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5	EY:ma DN:1. FICAT DESCE CAC His	at pe .471 CION RIPTI TGT Cys	A eptic METH (ON:S ACA Thr	ODS: SEQ I ACC Thr	GCA Ala 10	GTA Val	ATA Ile	Arg	Asn	Ile 15	Asn	48
	Asn 1 GAC	(i (x TTT Phe CAA	(A) FE (A) (E) (C) (C) (C) (C) (C) (C) (C) (C) (C) (C	EATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC	EY:ma DN:1. FICAT DESCR CAC His	at pe .471 TION RIPTI TGT Cys	METHON:SACA	ODS: SEQ I ACC Thr	GCA Ala 10 CAG	GTA Val CCT	ATA Ile GTG	Arg TTC	Asn GAG	Ile 15 GAT	Asn ATG	48
	Asn 1 GAC	(i (x TTT Phe CAA	(A) FE (A) (E) (C) (C) (C) (C) (C) (C) (C) (C) (C) (C	EATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5	EY:ma DN:1. FICAT DESCR CAC His	at pe .471 TION RIPTI TGT Cys	METHON:SACA	ODS: SEQ I ACC Thr	GCA Ala 10 CAG	GTA Val CCT	ATA Ile GTG	Arg TTC	Asn GAG Glu	Ile 15 GAT	Asn ATG	
	Asn 1 GAC Asp	(i (x TTT Phe CAA Gln	X) FE (I) (E) (C) (I) SE GGC Gly GTT Val	EATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIC ENTIFICE I CTT Leu 5 TTC Phe	EY:ma DN:1. FICAT DESCR CAC His GTT Val	at pe . 471 . 100 EIPTI TGT Cys GAC Asp	METHON:S ACA Thr AAA Lys	ODS: EQ I ACC Thr AGA Arg 25	GCA Ala 10 CAG Gln	GTA Val CCT Pro	ATA Ile GTG Val	Arg TTC Phe	Asn GAG Glu 30	Ile 15 GAT Asp	Asn ATG Met	96
	Asn 1 GAC Asp ACT	(i (x TTT Phe CAA Gln GAT	X) FE (I) (E) (C) (I) SE GGC Gly GTT Val	EATUR A) NAM B) LOO C) IDE CQUEN CGA Arg CTC Leu 20 GAT	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC	EY:ma DN:1. FICAT DESCR CAC His GTT Val	at pe . 471 TION TION TGT Cys GAC Asp	METHON:S ACA Thr AAA Lys AGT	IODS: BEQ I ACC Thr AGA Arg 25 GAA	GCA Ala 10 CAG Gln	GTA Val CCT Pro	ATA Ile GTG Val	Arg TTC Phe AGA	Asn GAG Glu 30 CTG	Ile 15 GAT Asp	Asn ATG Met ATA	
	Asn 1 GAC Asp ACT Thr	(i (x TTT Phe CAA Gln GAT Asp	X) FE (F	EATUR A) NAM B) LOC C) IDE EQUEN CGA Arg CTC Leu 20 GAT ASP	RE: ME/KE CATIC ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser	at per 1.471 CION CIPTI TGT Cys GAC Asp GCC Ala	METHON:SACA Thr AAA Lys AGT Ser	ACC Thr AGA Arg 25 GAA Glu	GCA Ala 10 CAG Gln CCC Pro	GTA Val CCT Pro CAG Gln	ATA Ile GTG Val ACC Thr	TTC Phe AGA Arg 45	Asn GAG Glu 30 CTG Leu	Ile 15 GAT Asp ATA Ile	Asn ATG Met ATA Ile	96
40	Asn 1 GAC Asp ACT Thr	(i (x TTT Phe CAA Gln GAT Asp	X) FE (A (E (C (I) SE GGC Gly GTT Val ATT Ile 35 TAC	EATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln	EY: ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser	at per 1.471 CION RIPTI TGT Cys GAC Asp GCC Ala	ACA Thr AAA Lys AGT Ser 40 GTA	ODS: SEQ I ACC Thr AGA Arg 25 GAA Glu	GCA Ala 10 CAG Gln CCC Pro	GTA Val CCT Pro CAG Gln	ATA Ile GTG Val ACC Thr	TTC Phe AGA Arg 45 GTG	Asn GAG Glu 30 CTG Leu ACC	Ile 15 GAT Asp ATA Ile	Asn ATG Met ATA Ile TCT	96
40	Asn 1 GAC Asp ACT Thr	(i (x TTT Phe CAA Gln GAT ASP ATG Met	X) FE (A (E (C (I) SE GGC Gly GTT Val ATT Ile 35 TAC	EATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIC ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln	EY: ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser	at per 1471 CION CIPTI TGT Cys GAC Asp GCC Ala GAA Glu	ACA Thr AAA Lys AGT Ser 40 GTA	ODS: SEQ I ACC Thr AGA Arg 25 GAA Glu	GCA Ala 10 CAG Gln CCC Pro	GTA Val CCT Pro CAG Gln	ATA Ile GTG Val ACC Thr GCT Ala	TTC Phe AGA Arg 45 GTG	Asn GAG Glu 30 CTG Leu ACC	Ile 15 GAT Asp ATA Ile	Asn ATG Met ATA Ile TCT	96
40	Asn 1 GAC Asp ACT Thr TAC Tyr	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50	X) FE (A (E (C (E)) SE GGC Gly GTT Val ATT Ile 35 TAC Tyr	CGA CTC Leu 20 CGA Arg CTC Leu 20 GAT Asp AAA Lys	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln GAC Asp	EY: ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser	at per 1.471 TION RIPTI TGT Cys GAC Asp GCC Ala GAA Glu 55	ACA Thr AAA Lys AGT Ser 40 GTA Val	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg	GCA Ala 10 CAG Gln CCC Pro GGA Gly	GTA Val CCT Pro CAG Gln CTG Leu	ATA Ile GTG Val ACC Thr GCT Ala 60	TTC Phe AGA Arg 45 GTG Val	Asn GAG Glu 30 CTG Leu ACC Thr	Ile 15 GAT Asp ATA Ile CTC Leu	Asn ATG Met ATA Ile TCT Ser	96 144 192
40 45	Asn 1 GAC Asp ACT Thr TAC Tyr GTG Val	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50 AAG	X) FE (A (E (C C C C C C C C C C C C C C C C C C	EATUR A) NAM B) LOC C) IDE CQUEN CGA Arg CTC Leu 20 GAT ASP AAA Lys	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser	at per 1471 TION RIPTI TGT Cys GAC Asp GCC Ala GAA Glu 55 TCT	ACA Thr AAA Lys AGT Ser 40 GTA Val	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg	GCA Ala 10 CAG Gln CCC Pro GGA Gly	GTA Val CCT Pro CAG Gln CTG Leu	ATA Ile GTG Val ACC Thr GCT Ala 60 AAG	TTC Phe AGA Arg 45 GTG Val	Asn GAG Glu 30 CTG Leu ACC Thr	Ile 15 GAT Asp ATA Ile CTC Leu	Asn ATG Met ATA Ile TCT Ser ATT	96
40	Asn 1 GAC Asp ACT Thr TAC Tyr GTG Val 65	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50 AAG Lys	X) FE (A (E (C (E)) SE GGC Gly GTT Val ATT Ile 35 TAC Tyr GAT Asp	CATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln GAC Asp	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser ATG Met 70	CDNA at personal services of the services of t	ACC Thr	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg CTC Leu	GCA Ala 10 CAG Gln CCC Pro GGA Gly TCC Ser	GTA Val CCT Pro CAG Gln CTG Leu TGT Cys 75	ATA Ile GTG Val ACC Thr GCT Ala 60 AAG Lys	Arg TTC Phe AGA Arg 45 GTG Val AAC Asn	Asn GAG Glu 30 CTG Leu ACC Thr AAG Lys	Ile 15 GAT Asp ATA Ile CTC Leu ATC	Asn ATG Met ATA Ile TCT Ser ATT Ile 80	96 144 192 240
40 45	Asn 1 GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50 AAG Lys	X) FE (A (E (C (E)) SE GGC Gly GTT Val ATT Ile 35 TAC Tyr GAT Asp GAG	CATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO CENTIFICE I CTT Leu 5 TTC Phe CAA Gln GAC Asp AAA Lys	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser ATG Met 70 GAT	CDNA At pe 1.471 CION RIPTI TGT Cys GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA	ACA Thr AAA Lys AGT Ser 40 GTA Val ACC Thr	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg CTC Leu	GCA Ala 10 CAG Gln CCC Pro GGA Gly TCC Ser	GTA Val CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT	ATA Ile GTG Val ACC Thr GCT Ala 60 AAG Lys	Arg TTC Phe AGA Arg 45 GTG Val AAC Asn	Asn GAG Glu 30 CTG Leu ACC Thr AAG Lys	Ile 15 GAT Asp ATA Ile CTC Leu ATC Ile	Asn ATG Met ATA Ile TCT Ser ATT Ile 80 AGT	96 144 192
40 45	Asn 1 GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50 AAG Lys	X) FE (A (E (C (E)) SE GGC Gly GTT Val ATT Ile 35 TAC Tyr GAT Asp GAG	CATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO ENTIF NCE I CTT Leu 5 TTC Phe CAA Gln GAC Asp	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser ATG Met 70 GAT	CDNA At pe 1.471 CION RIPTI TGT Cys GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA	ACA Thr AAA Lys AGT Ser 40 GTA Val ACC Thr	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg CTC Leu	GCA Ala 10 CAG Gln CCC Pro GGA Gly TCC Ser	GTA Val CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT	ATA Ile GTG Val ACC Thr GCT Ala 60 AAG Lys	Arg TTC Phe AGA Arg 45 GTG Val AAC Asn	Asn GAG Glu 30 CTG Leu ACC Thr AAG Lys	Ile 15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln	Asn ATG Met ATA Ile TCT Ser ATT Ile 80 AGT	96 144 192 240
40 45	Asn 1 GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe	X) FE (A (E (C (E	CATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO CATIO CTT Leu 5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser ATG Met 70 GAT ASP	at per 1.471 TION RIPTI TGT Cys GAC Asp GCC Ala GLU 55 TCT Ser CCA Pro	ACA Thr AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu	GCA Ala 10 CAG Gln CCC Pro GGA Gly TCC Ser AAT Asn 90 CCA	GTA Val CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile	ATA Ile GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp	Arg TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp	Asn GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile	Ile 15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG	ASN ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser GAG	96 144 192 240
40 45	Asn 1 GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT	(i (x TTT Phe CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe	X) FE (A (E (C (E	CATURAL NAME OF THE PROPERTY O	RE: ME/KE CATIO CENTIFICE I CTT Leu 5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85	EY:ma DN:1. FICAT DESCR CAC His GTT Val AGT Ser AGT Ser ATG Met 70 GAT ASP	at per 1.471 TION RIPTI TGT Cys GAC Asp GCC Ala GLU 55 TCT Ser CCA Pro	ACA Thr AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro	IODS: SEQ I ACC Thr AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu	GCA Ala 10 CAG Gln CCC Pro GGA Gly TCC Ser AAT Asn 90 CCA	GTA Val CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile	ATA Ile GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp	Arg TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp	Asn GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile	Ile 15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG	ASN ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser GAG	96 144 192 240 288

	TTT Phe	GAA Glu	TCT Ser 115	TCA Ser	CTG Leu	TAT Tyr	GAA Glu	GGA Gly 120	CAC His	TTT Phe	CTT Leu	GCT Ala	AGC Ser 125	Glr	AAC Lys	GAA Glu	384
5	Asp	Asp 130	Aia	Phe	Lys	Leu	Ile 135	CTG Leu	Lys	Lys	Lys	Asp	GAA Glu	AAT ASD	GGG Gly	GAT Asp	432
	AAA Lys 145	TCT Ser	GTA Val	ATG Met	TTC Phe	ACT Thr 150	CTC Leu	ACT Thr	AAC Asn	TTA Leu	CAT His	Gln	AGT Ser				471
10																	
15	(25)	(: (:	(E (C (I (ii) MC (iX) FE	QUEN A) LE 3) TY C) ST C) TO DLEC EATU	CE CI NGTH PE: ni RANDI POLO ULE :	HARAGE 69 1 UCLE 1 EDNE: GY:1: TYPE	CTER: base ic ac SS:do inea: :Othe	ISTIC pair cid ouble r er no	CS: rs e ucle:	ic a	cid						
20						EY:s: ON:1		eptio	ie								
20		()		C) ID	ENTI	FICA	NOI	METH S: NOI			0:24	:					
	ATG(GCCTT GTGGC	rga c	CTT	rgct:	A TI	CTGG1	rggc	CTO	CTG	GTGC	TCA	GCTG	CAA	GTCA	AGCTGC	60
25																	69
30	(26)	(i	(E (C (E (i)MC (i)OR	QUENC () LEN () TYN () STN () TON () LECU () LECU	CE CH NGTH: PE: nu RANDE POLOC JLE T NAL S	HARAC : 471 : 471 : Clei EDNES GY: li TYPE:	DETERI base c ac Ss:do near cDNA	STICE pai	:: :rs								
35		, :	(F	TIS	SUE	TYPE		rer									
			(B) NAN) LOC !) IDE	E/KE CATIO ENTIF	N:1. ICAT	.471 'ION	METH	ODS:		JO • 2 9	· •					
40									-								
	Tyr 1	Phe	GGC Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn	48
45	GAC Asp	CAA Gln	GTT Val	CTC Leu 20	TTC Phe	ATT Ile	GAC Asp	CAA Gln	GGA Gly 25	AAT Asn	CGG Arg	CCT Pro	CTA Leu	TTT Phe 30	GAA Glu	GAT Asp	96
	ATG Met	ACT Thr	GAT Asp 35	TCT	GAC Asp	TGT Cys	AGA Arg	GAT Asp 40	AAT	GCA Ala	CCC Pro	CGG Arg	ACC Thr 45	ATA	TTT Phe	ATT Ile	144
50	ATA Ile	AGT Ser 50	ATG Met	TAT Tyr	AAA Lys	GAT Asp	AGC Ser 55	CAG Gln	CCT Pro	AGA Arg	GGT Gly	ATG Met 60	GCT	GTA Val	ACT Thr	ATC Ile	192
	TCT Ser 65	GTG	AAG Lys	TGT Cys	GAG Glu	AAA Lys 70	ATT	TCA Ser	ACT Thr	CTC Leu	TCC Ser 75	TGT	GAG Glu	AAC Asn	AAA Lys	ATT Ile 80	240
55	ATT	TCC Ser	TTT . Phe	AAG Lys	GAA Glu	ATG	AAT Asn	CCT Pro	CCT Pro	GAT Asp	AAC	ATC Ile	AAG Lys	GAT Asp	ACA Thr	AAA	288

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85
                                           90
     AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAT AAG
     Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                  100
                                       105
                                                            110
     ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TGT GAA
                                                                            384
     Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
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                                   120
                                                       125
     AAA GAG AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG
                                                                            432
     Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
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                              135
                                                   140
     GGG GAT AGA TCT ATA ATG TTC ACT GTT CAA AAC GAA GAC
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     (27) INFORMATION FOR SEQ ID NO:26:
            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 570 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
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            (ii) MOLECULE TYPE: cDNA
            (iX) FEATURE:
                (A) NAME/KEY: 5' UTR
                (B) LOCATION: 1..15
                (C) IDENTIFICATION METHODS:S
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                (A) NAME/KEY: sig peptide
                (B) LOCATION: 16..84
                (C) IDENTIFICATION METHODS:S
                (A) NAME/KEY: mat peptide
                (B) LOCATION: 85..555
                (C) IDENTIFICATION METHODS:S
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                (A) NAME/KEY: 3' UTR
                (B) LOCATION: 556..570
                (C) IDENTIFICATION METHODS:S
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
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     ACACCTCGAG CCACC ATG GCC TTG ACC TTT GCT TTA CTG GTG GCC CTC CTG
                       Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu
                                   -20
                                                        -15
     GTG CTC AGC TGC AAG TCA AGC TGC TCT GTG GGC TAC TTT GGC AAG CTT
     Val Leu Ser Cys Lys Ser Ser Cys Ser Val Gly Tyr Phe Gly Lys Leu
                              - 5
40
     GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT GAC CAA GTT CTC TTC
                                                                           147
     Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe
                      10
                                           15
     ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT ATG ACT GAT TCT GAC
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     Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp
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                                      30
                                                           35
     TGT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT ATA AGT ATG TAT AAA
                                                                           243
     Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys
             40
                                  45
     GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC TCT GTG AAG TGT GAG
                                                                           291
     Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Cys Glu
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                              60
                                                   65
     AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT ATT TCC TTT AAG GAA
                                                                           339
     Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu
                          75
                                               80
     ATG AAT CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC ATC ATA TTC
                                                                           387
     Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe
                                          95
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	TTT Phe	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT Asn	AAG Lys	ATG Met	CAA Gln	TTT Phe 115	GAA Glu	TCT Ser	435
5	TCA Ser	TCA Ser	TAC Tyr 120	GAA	GGA Gly	TAC Tyr	TTT Phe	CTA Leu 125	GCT	TGT Cys	GAA Glu	AAA Lys	Glu	AGA	GAC Asp	CTT Leu	483
	TTT Phe	AAA Lys 135	CTC	ATT Ile	TTG Leu	AAA Lys	AAA Lys 140	GAG	GAT Asp	GAA Glu	TTG Leu	GGG Gly 145	GAT Asp	AGA Arg	TCT Ser	ATA Ile	531
10		TTC			CAA Gln		GAA		TAG	GCGG	CCG	CGTG	r				570
15	(28)	(:	i) SE(() (1 (1	QUENC A) LEI B) TY: D) TO:	N FOI CE CI NGTH PE:ar POLOC ULE T	HARAG :10 a nino GY:1:	CTER: amino acio inea:	ISTIC o ac: i c	CS:								
20		(1	v) FR	AGME	NT TY NCE I	(PE:1	V-te	rmina				:					
	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10							
25	(29)		i) SE([]	QUENC A) LEI	N FOR CE CH NGTH: PE:nu	IARA(:471	TER!	STIC pai	CS:								
30			(I Li) M(Vi) OF (<i>I</i>	O) TOI OLECT RIGII A) ORG	RANDE POLOC JLE 1 NAL S GANIS SSUE	SY:1i TYPE: SOUR(SM:mo	inear :cDN/ CE: ouse	A A	2								
35			LX) FI (1 (E	EATUR A) NAN B) LOC C) IDE		EY:ma N:1. FICAT	at pe	eptic L METH	ODS :		10 : 28	3:					
40												ATA Ile					48
45	GAC									CAG		GTG Val			GAT		96
	Thr	Asp	Ile 35	Asp	Gln	Ser	Ala	Ser 40	Glu	Pro	Gln	ACC Thr	Arg 45	Leu	Ile	Ile	144
50	Tyr	Met 50	Tyr	Lys	Asp	Ser	Glu 55	Val	Arg	Gly	Leu	GCT Ala 60	Val	Thr	Leu	Ser	192
	Val 65	Lys	Asp	Ser	Lys	Met 70	Ser	Thr	Leu	Ser	Cys 75	AAG Lys	Asn	Lys	Ile	Ile 80	240
55	TCC Ser	TTT Phe	GAG Glu	GAA Glu	ATG Met	GAT Asp	CCA Pro	CCT Pro	GAA Glu	AAT Asn	ATT Ile	GAT Asp	GAT Asp	ATA Ile	CAA Gln	AGT Ser	288

	GAT CTC ATZ	ነ ጥጥር ጥጥጥ ር ል	ב אאא רכיד מדי	CCA GGA CAC	AAC AAG ATG GAG	226
	Asp Leu Ile	Phe Phe Gl:	n Lys Arg Val 105	Pro Gly His	Asn Lys Met Glu	336
5	TTT GAA TCT Phe Glu Ser	Ser Leu Ty	r GAA GGA CAC r Glu Gly His 120	TTT CTT GCT Phe Leu Ala	TGC CAA AAG GAA Cys Gln Lys Glu 125	384
	GAT GAT GCT Asp Asp Ala	TTC AAA CT	C ATT CTG AAA 1 Ile Leu Lys	Lys Lys Asp	GAA AAT GGG GAT Glu Asn Gly Asp	432
10	130 AAA TCT GTA Lys Ser Val 145	A ATG TTC AC Met Phe Th: 150	r Leu Thr Asn	TTA CAT CAA Leu His Gln 155	AGT Ser	471
15	(i)SE ((ATION FOR SI QUENCE CHARA A) LENGTH: 570 B) TYPE: nucle C) STRANDEDNI	ACTERISTICS: D base pairs eic acid ESS:double			
20	(ii) M (iX) F ((D) TOPOLOGY: 1 IOLECULE TYPE EATURE: A) NAME/KEY: 5 B) LOCATION: 1 C) IDENTIFICE	E:cDNA 5' UTR	:S		
25	((((A) NAME/KEY: SB) LOCATION: SC) IDENTIFICA A) NAME/KEY: TB) LOCATION: SC	sig peptide .684 ATION METHODS mat peptide 85555	: S		
30	((A) NAME/KEY: 3 B) LOCATION: S C) IDENTIFICA		:S		
35	ACACCTCGAG	CCACC ATG GC Met Al	CC TTG ACC TT a Leu Thr Pho -20	GCT TTA CTG	GTG GCC CTC CTG Val Ala Leu Leu -15	51
	GTG CTC AGC Val Leu Ser -10	TGC AAG TCA Cys Lys Ser	AGC TGC TCT Ser Cys Ser -5	GTG GGC AAC TVal Gly Asn I	TTT GGC CGA CTT Phe Gly Arg Leu 5	99
40	His Cys Thr	Thr Ala Val	Ile Arg Asn	Ile Asn Asp (CAA GTT CTC TTC Gln Val Leu Phe 20	147
	GTT GAC AAA Val Asp Lys	AGA CAG CCT Arg Gln Pro	GTG TTC GAG Val Phe Glu 30	GAT ATG ACT C Asp Met Thr A	SAT ATT GAT CAA Asp Ile Asp Gln 35	195
45	AGT GCC AGT Ser Ala Ser 40	GAA CCC CAG Glu Pro Gln	ACC AGA CTG Thr Arg Leu 45	Ile Ile Tyr N	ATG TAC AAA GAC Met Tyr Lys Asp	243
	AGT GAA GTA Ser Glu Val	AGA GGA CTG Arg Gly Leu	GCT GTG ACC Ala Val Thr 60	CTC TCT GTG A	AAG GAT AGT AAA Lys Asp Ser Lys	291
50	ATG TCT ACC	CTC TCC TGT Leu Ser Cys	AAG AAC AAG	ATC ATT TCC T	CTT GAG GAA ATG Phe Glu Glu Met	339
55	GAT CCA CCT	GAA AAT ATT	GAT GAT ATA Asp Asp Ile	CAA AGT GAT G Gln Ser Asp I	85 CTC ATA TTC TTT Seu Ile Phe Phe	387
55	CAG AAA CGT		CAC AAC AAG	95 ATG GAG TTT (AA TCT TCA CTG	435

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Gln Lys Arg Val Pro Gly His Asn Lys Met Glu Phe Glu Ser Ser Leu
                  105
                                        110
     TAT GAA GGA CAC TTT CTT GCT TGC CAA AAG GAA GAT GAT GCT TTC AAA
                                                                             483
     Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu Asp Asp Ala Phe Lys
5
              120
                                   125
                                                        130
     CTC ATT CTG AAA AAA AAG GAT GAA AAT GGG GAT AAA TCT GTA ATG TTC
                                                                             531
     Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp Lys Ser Val Met Phe
          135
                               140
                                                    145
     ACT CTC ACT AAC TTA CAT CAA AGT TAGGCGGCCG CGTGT
                                                                             570
10
     Thr Leu Thr Asn Leu His Gln Ser
      (31) INFORMATION FOR SEO ID NO:30:
            (i) SEQUENCE CHARACTERISTICS:
15
                (A) LENGTH:6 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: peptide
            (v) FRAGMENT TYPE: N-terminal fragment
20
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:
     Asn Phe Gly Arg Leu His
```

Claims

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 An isolated polypeptide which is capable of inducing the production of interferon-gamma by immunocompetent cells, said polypeptide containing either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs:1-3 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine(s);

- 2. The polypeptide of claim 1, wherein said different amino acid(s) is one or more amino acids selected from the group consisting of serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine.
- 55 3. The polypeptide of claim 1, wherein the amino acid sequence wherein one or more cysteines are replaced with deferent amino acid(s) is derived from the amino acid sequence of SEQ ID NO:4, containing SEQ ID NOs:1-3 as consensus sequences;

SEQ ID NO: 4:

5	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn
	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glu	Asp
10	Met	Thr	Asp 35	Ser	Asp	Cys	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr 45	Ile	Phe	Ile
	Ile	Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile
0_	Ser 65	Val	Lys	Cys	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Cys	Glu	Asn	Lys	Ile 80
15	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	
	Ser	Asp	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys
20	Met	Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu
	Lys	Glu 130	Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu	Asp	Glu	Leu
25	Gly 145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp.	,		

4. The polypeptide of claim 1, wherein the amino acid sequence wherein one or more cysteines replaced with different amino acid(s) is derived from the amino acid sequence of SEQ ID NO:5, containing SEQ ID NOs:1-3 as consensus sequences:

SEQ ID NO: 5:

30

35	Asn 1	Phe	Gly	Arg	Leu 5	His	Cys	Thr	Thr	Ala 10	Val	Ile	Arg	Asn	Ile 15	Asn
	Asp	Gln	Val	Leu 20	Phe	Val	Asp	Lys	Arg 25	Gln	Pro	Val	Phe	Glu 30	Asp	Met
	Thr	Asp	Ile 35	Asp	Gln	Ser	Ala	Ser 40	Glu	Pro	Gln	Thr	Arg 45	Leu	Ile	Ile
40	Tyr	Met 50	Tyr	Lys	Asp	Ser	Glu 55	Val	Arg	Gly	Leu	Ala 60	Val	Thr	Leu	Ser
	Val 65	Lys	Asp	Ser	Lys	Met 70	Ser	Thr	Leu	Ser	Cys 75	Lys	Asn	Lys	Ile	Ile 80
45	Ser	Phe	Glu	Glu	Met 85	Asp	Pro	Pro	Glu	Asn 90	Ile	Asp	Asp	Ile	Gln 95	Ser
	Asp	Leu	Ile	Phe 100	Phe	Gln	Lys	Arg	Val 105	Pro	Gly	His	Asn	Lys 110	Met	Glu
	Phe	Glu	Ser 115	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Cys 125	Gln	Lys	Glu
50	Asp	Asp 130	Ala	Phe	Lys	Leu	Ile 135	Leu	Lys	Lys	Lys	Asp 140	Glu	Asn	Gly	Asp
	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser.			

55 The polypeptide of claim 1, which contains an amino acid sequence selected from the group consisting of SEQ ID NO:6-12, derived from the amino acid sequence of SEQ ID NO:4;

SEQ ID NO: 6:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 5 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 15 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 125 20 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

25 SEQ ID NO: 7:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 30 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 35 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 40 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

SEQ ID NO: 8:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile

35 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 5 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 10 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 15 SEQ ID NO: 9: Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 20 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 25 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 30 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 100 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 35 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150 SEQ ID NO: 10: 40 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 45 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile 50 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 5 150 SEQ ID NO: 11: Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 15 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 20 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 25 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 145 150 **155**. 30 SEQ ID NO: 12: Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 35 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 40 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 45 100 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 115 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 50 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp.

^{6.} The polypeptide of claim 1, which contains an amino acid sequence selected from the group consisting of SEQ ID NO:13 and 14, derived from the amino acid sequence of SEQ ID NO:5;

SEQ ID NO: 13:

- Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn 5 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 10 55 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 75 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 15 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 105 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu 120 20 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 135 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 145 150 155 25 SEQ ID NO: 14: Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 30 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 55 35 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 70 75 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 40 105 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Ser Gln Lys Glu 120 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 135 45 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser. 145 150 155
- 7. The polypeptide of claims 1, which additionally has one or more properties selected from the group consisting of enhancing cytotoxicities of killer cells and inducing formation of killer cells.
 - 8. The polypeptide of claim 3, which additionally has one or more properties selected from the group consisting of enhancing cytotoxicity of killer cells and inducing formation of killer cells.
- 9. A DNA encoding the polypeptide of claim 1.
 - 10. The DNA of claim 9, which contains a nucleotide sequence selected either from the group consisting of the nucleotide sequences of SEQ ID NOs:15-23 and their complementary nucleotide sequences, or from the other group.

consisting of the nucleotide sequences derived from one of the nucleotide sequences of the former group by replacing one or more of the nucleotides with different one(s) without altering the amino acid sequences encoded thereby;

	SEQ	ID !	ю:	15:													
		TTT Phe								Ser					Leu		48
10		CAA Gln															96
		ACT Thr															144
15	ATA	AGT Ser	35 ATG	TAT	AAA	GAT	AGC	40 CAG	CCT	AGA	GGT	ATG	45 GCT	GTA	ACT	ATC	192
	TCT	50 GTG	AAG	TCT	GAG	AAA	55 att	TCA	ACT	CTC	TCC	60 TGT	GAG	AAC	AAA	ATT	240
20	65	Val TCC				70					75				_	80	200
	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	288
25	AGT Ser	GAC Asp	ATC	ATA Ile 100	TTC Phe	TTT	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT Asn	AAG Lys	336
	ATG Met	CAA Gln	TTT Phe 115	GAA	TCT Ser	TCA Ser	TCA Ser	TAC Tyr 120	GAA	GGA Gly	TAC Tyr	TTT Phe	CTA Leu 125	GCT	TGT Cys	GAA Glu	384
30		GAG Glu 130	AGA					CTC					GAG				432
35		GAT Asp					TTC					GAA					471
. •																	

SEQ ID NO: 16:

	TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
5																Asn	
J	1		_	-	5			•		10			3		15		
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	СТА	ጥጥጥ		GAT	96
																Asp	90
		U		20			nsp	01	25		9		Deu	30	GIU	ush	
	ልጥር	ልርጥ	САТ		GAC	שרש	AGA	CAT		GCA	CCC	ccc	NCC		an en en	ATT	1.4.4
10																	144
	Mec	1111		Ser	vəb	261	Arg		ASII	WIG	PIO	Arg		TTE	Pne	Ile	
	አመል	A Cm	35	mam		~ m	100	40	CCM				45				
																ATC	192
	116		Met	TYT	гĀг	Asp		GIN	Pro	Arg	GTĀ		Ala	Val	Thr	Ile	
	m.c.m	50					55					60					
15																ATT	240
		vai	Lys	Ser	Glu		Ile	Ser	Thr	Leu		Cys	Glu	Asn	Lys	Ile	
	65					70					75					80	
																AAA	288
	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
20					85					90					95		
20																AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
				100					105					110		_	
	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
25			115					120		_	_		125		•		
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
	_	130	_	_			135				-	140					
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
	_											Glu					-,-
30	145	•	-			150	-				155						
	SEO	ID 1	NO: 1	7:													
	TAC	ጥጥጥ	GGC	AAG	СТТ	GAA	TCT	ΔΔΔ	ጥጥ A	TCA	GTC	ATA	AGA	די א מ	ጥጥር	יי א א	48
35												Ile					40
	1	1	0-1	_, _	5	014		1 73	LCu	10	¥01	116	Arg	YOU	15	ASII	
	_	CAA	CTT	כיייכ	ጥጥር	Δጥጥ	GAC	CAA	GGA		CGG	CCT	רידיא	արարա		CAT	96
												Pro					90
	1100	01	V 4.1	20	1	116	nap	G111	25	ASII	ALG	FLO	Leu	30	GIU	MSD	
	ATC	АСТ	CAT		GAC	ጥርጥ	ACA	CAT		CCA	CCC	CGG	NCC.		തനാ	N COUTO	1 4 4
40												Arg					144
	1100	****	35 ¹	Ser	nsp	Cys	Arg		VOII	VIG	PIU	Arg		116	FILE	TTE	
	አጥአ	λ Cm		ጥልጥ	222	CAT	100	40	CCM	202	CCM	ATG	45 CCD	cm.	3 O.M.	1 ma	100
	AIA	AG1	Mat	TWI	AAA	GAI	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	TTE		Met	TYL	μλε	ASP		OID	Pro	Arg	GIY	Met	Ala	var	Thr	TTE	
45	mam	50			010		55					60					
43	TCT	GIG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240
		val	rys	Ser	GIU		TTE	Ser	Thr	Leu		Cys	Glu	Asn	Lys		
	65					70					75					80	
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
	Ile	Ser	Phe	Lys		Met	Asn	Pro	Pro		Asn	Ile	Lys	Asp		Lys	
50					85					90					95		
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	Ser	Asp			Phe	Phe	Gln	Arg		Val	Pro	Gly			Asn	Lys	
				100				_	105					110			
	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TCT	GAA	384

	Met	Gln	Phe		Ser	Ser	Ser	Tyr 120		Gly	Туг	Phe	Leu 125		Ser	Glu	
	AAA	GAG			CTT	ттт	. AAA			ጥጥር		ААА			GAA	TTG	432
5	Lys	Glu 130	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	432
	ccc			тст	ልጥል	ልጥር	135 ייייי	ACT	ርጥጥ	CAA	220	140					471
								Thr									471
	145		· · 9			150			,	V	155						
10	SEQ	ID	NO:	18:													
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		Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
	1	~~~		c.m.c	5		~~~			10					15		
15															GAA		96
	vəħ	G111	Val	20	FIIE	116	vəh	GIII	25	ASII	Arg	PIO	теп	30	Glu	Asp	
	ATG	ACT	GAT		GAC	TCT	AGA	GAT		GCA	ccc	CGG	ACC		TTT	ATT	144
															Phe		
			35				_	40					45				
20															ACT		192
	тте	Ser 50	Met	туг	rys	Asp		GIn	Pro	Arg	Gly		Ala	Val	Thr	Ile	
	ጥርጥ		AAG	ጥርጥ	GAG	272	55	ጥ ር እ	A CIT	CTC	ሞርር	60 #6#	CAC	3 3 C	AAA	3 mm	240
															Lys		240
05	65		-10			70			****		75	O _I S	014	A3II	БyЗ	80	
25	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
	Ile	Ser	Phe	Lys		Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
					85					90					95		
															AAT		336
30	261	vah	116	100	FILE	FIIE	GIII	Arg	105	AGI	PIO	GTÅ	nis	110	Asn	гĀ2	
	ATG	CAA	TTT		TCT	TCA	TCA	TAC		GGA	TAC	ттт	СТА		TCT	GAA	384
	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu	004
			115					120			_		125				
															GAA		432
35	Lys		Arg	Asp	Leu	Phe		Leu	Ile	Leu	Lys		Glu	Asp	Glu	Leu	
	GGG	130	ACA	ጥርጥ	מ ידי מ	እ ጥር	135	ACT	CTT	CAA	እአሮ	140	CAC				471
								Thr									471
	145		5			L50			. •		155						
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	Asp	GIn	vai		Phe	Ile	Asp	Gln		Asn	Arg	Pro	Leu		Glu	Asp	
	A TC	а С Ф	CAT	20 TCT	CNC	mcm	202	CAM	25	CCA	CCC	CCC	200	30	ттт	.	2.4.4
	Met	Thr	Acn	Ser	Asn	Ser	ATA	Pen	VVI	Ala	Pro	220	Th≻	TIA	Phe	ATT	144
			35		5		9	40		-120	0	9	45	E	* IIE	116	
50	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
	ma~	50		ma-i	~ . ~		55 NOT					60					
	TCT	GIG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TUT	GAG	AAC	AAA	ATT	240

	Ser 65	Val	Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Ser	Glu	Asn	Lys	ile 80	
5	ATT Ile	TCC Ser	TTT	AAG Lys	GAA Glu 85	ATG Met	AAT	CCT Pro	CCT Pro	GAT Asp 90	AAC	ATC Ile	AAG Lys	GAT Asp	ACA Thr	AAA Lys	288
	AGT Ser	GAC Asp	ATC Ile	ATA Ile 100	TTC Phe	TTT Phe	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT Asn	AAG Lys	336
10	ATG Met	CAA Gln	TTT Phe 115	GAA Glu	TCT	TCA Ser	TCA Ser	TAC Tyr 120	GAA Glu	GGA	TAC	TTT	CTA Leu 125	GCT Ala	TCT	GAA Glu	384
	AAA Lys	GAG Glu 130	Arg	GAC Asp	CTT Leu	TTT Phe	AAA Lys 135	CTC	ATT	TTG Leu	AAA Lys	AAA Lys 140	GAG Glu	GAT	GAA Glu	TTG Leu	432
15		GAT Asp										GAA Glu	GAC				471
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25	GAC Asp	CAA Gln	GTT Val	CTC Leu 20	TTC Phe	ATT Ile	GAC Asp	CAA Gln	GGA Gly 25	AAT Asn	CGG Arg	CCT Pro	CTA Leu	TTT Phe 30	GAA	GAT Asp	96
25	ATG Met	ACT	GAT Asp 35	TCT	GAC Asp	TCT Ser	AGA Arg	GAT Asp 40	AAT	GCA Ala	CCC Pro	CGG Arg	ACC Thr 45	ATA	TTT Phe	ATT Ile	144
30	ATA Ile	AGT Ser 50	ATG	TAT Tyr	AAA Lys	GAT Asp	AGC Ser 55	CAG	CCT Pro	AGA Arg	GGT Gly	ATG Met 60	GCT	GTA Val	ACT Thr	ATC Ile	192
	TCT Ser 65	GTG Val	AAG Lys	TCT Ser	GAG Glu	AAA Lys 70	ATT	TCA Ser	ACT Thr	CTC Leu	TCC Ser 75	GCT	GAG Glu	AAC Asn	AAA Lys	ATT Ile 80	240
35	ATT	TCC Ser	TTT Phe	AAG Lys	GAA Glu 85	ATG	AAT Asn	CCT Pro	CCT Pro	GAT Asp 90	AAC	ATC Ile	AAG Lys	GAT Asp	ACA Thr 95	AAA	288
	AGT Ser	GAC Asp	ATC Ile	ATA Ile 100	TTC Phe	TTT Phe	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT	AAG Lys	336
40	ATG Met	CAA Gln	TTT Phe 115	GAA Glu	TCT Ser	TCA Ser	TCA Ser	TAC Tyr 120	GAA	GGA Gly	TAC Tyr	TTT Phe	CTA Leu 125	GCT	TGT Cys	GAA Glu	384
	AAA Lys	GAG Glu 130	AGA Arg	GAC Asp	CTT Leu	TTT Phe	AAA Lys 135	CTC Leu	ATT	TTG Leu	AAA Lys	AAA Lys 140	GAG	GAT Asp	GAA Glu	TTG Leu	432
45		GAT Asp															471
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		CAA	GTT	CTC		ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	15 GAA	GAT	96

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5				Ser					Asn					Ile		Ile	
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10	TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
10	65					70					75	i			-	Ile 80	
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
					85					90		Ile		_	95	_	
15																AAG	336
				100					105					110		Lys	
												TTT					384
20			115					120				Phe	125				
												AAA					432
		130					135					Lys 140		Asp	Glu	Leu	
												GAA Glu					471
25	145	vaħ	n. y	361	116	150	rne	TIIL	AGT	GIII	155	Giu	ASD				
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30	Asn 1	Phe	Gly	Arg	Leu 5	His	Ala	Thr	Thr	Ala 10	Val	Ile	Arg	Asn	Ile 15	Asn	
												GTG					96
				20			_	_	25			Val		30	-		
05												ACC					144
35			35					40				Thr	45				
												GCT Ala					192
		50		_	_		55			_		60					240
40	Val	Luc	ACD	Cor	Luc	Mot	COT	Th-	LOV	SOF	Cuc	AAG Lys	AAC	AAG	ATC	ATT	240
	65			Ser			Ser		Deu				MSII	гÃ2		80	
			GAG	GAA	ATG		CCA	ССТ	GAA	AAT		GAT	GAT	АТА			288
												Asp					200
45	GAT	CTC	ATA	TTC	TTT	CAG	AAA	CGT	GTT	CCA	GGA	CAC	AAC	AAG	ATG	GAG	336
				100				_	105			His		110			
												GCT					384
50	Phe	Glu	Ser 115	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Cys 125	Gln	Lys	Glu	
•	GAT	GAT	GCT	TTC	AAA	CTC	ATT	CTG	AAA	AAA	AAG	GAT	GAA	AAT	GGG	GAT	432
	Asp	Asp 130	GCT Ala	Phe	Lys	Leu	Ile 135	CTG Leu	Lys	Lys	Lys	GAT Asp 140 CAA	GAA Glu	AAT Asn	GGG Gly	GAT Asp	432

	Lys 145		Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser				
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15			TAC Tyr														192
			GAT Asp														240
20			GAG Glu														288
25	Asp	Leu	ATA Ile	Phe 100	Phe	Gln	Lys	Arg	Val 105	Pro	Gly	His	Asn	Lys 110	Met	Glu	336
	Phe	Glu	TCT Ser 115	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Ser 125	Gln	Lys	Glu	384
30			GCT Ala														432
			GTA Val														471
35	11. The	DNA	of clair	n 9, wh	nich co	ntains	the nu	cleotic	le sequ	ence	of SEC	ID NO	D:24 a	t the 5'	-termir	nus.	
	SEQ	ID 1	10: 2	24:													
40		CCTT TGG(CTTI	rgcti	T AC	TGGT	rGGC	C CTC	CTG	STGC	TCAC	CTG	CAA (GTCA	AGCTGC	60 69
	12. The									·		le vect	or.				
45	13. The													:- 4 :	-4		- 'Ab - 1' - 1
			or clai , and -h				nost	s a ce	III Sele	ctea ir	om ine	e grou	p cons	isting	or mar	nmalian-e _l	oitnellai,
50	15 . A p										•						
		an ap	ing a co propria ting the	te hos	t, to pr	oduce	a poly	peptid	e, and			im 1, c	btaina	ble by	introdu	ucing the D	NA into

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interstitial, and -hematopoietic cells.

16. The process of claim 15, wherein said host is a cell selected from the group consisting of mammalian-epithelial, -

- 17. The process of claim 15, wherein said polypeptide is collected by one or more techniques selected from the group consisting of dialysis, salting out, filtration, concentration, fractional precipitation, ion-exchange chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis, and isoelectric focusing gel electrophoresis.
- 18. The process of claim 15, wherein said polypeptide is collected by an immunoaffinity chromatography using a monoclonal antibody.
- 19. An agent for susceptive diseases, which contains the polypeptide of claim 1 as an effective ingredient.
- 20. The agent of claim 19, which additionally contains interleukin 2.

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- 21. The agent of claim 19, which contains a serum albumin, gelatin, a saccharide, or a buffer as a stabilizer.
- 22. The agent of claim 19, which is in the form of an antitumor agent.
 - 23. The agent of claim 22, which is in the form of an antitumor immunotherapeutic agent.
 - 24. The agent of claim 19, which is in the form of an antiviral agent.
 - 25. The agent of claim 19, which is in the form of an antimicrobial agent.
 - 26. The agent of claim 19, which is in the form of an anti-immunopathic agent.
- 25 27. The agent of claim 26, which additionally contains interleukin 12.
 - 28. The agent of claim 26, which is for treating atopic diseases.

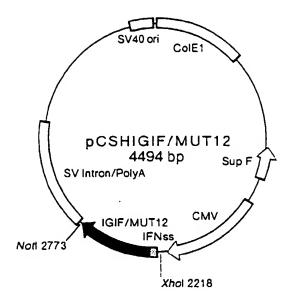


FIG. 1

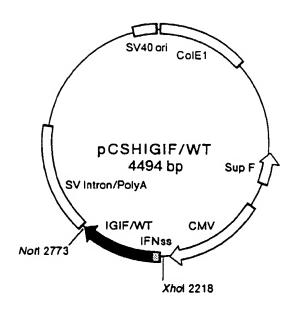
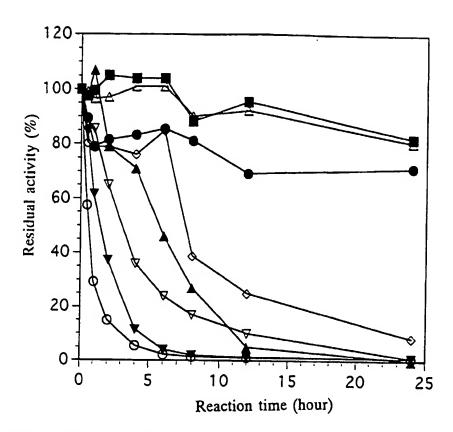


FIG. 2



Note: In the figure, the symbol "O—O" shows the time course upon the activity of a wild-type polypeptide; and the symbols "▼—▼", "▲—▲", "▼—▼", "♦—◆", "●—●", "■—■", and "△—△" show the time course upon the activity of the present polypeptides obtained by the methods in Examples A-1 to A-7, respectively.

FIG. 3

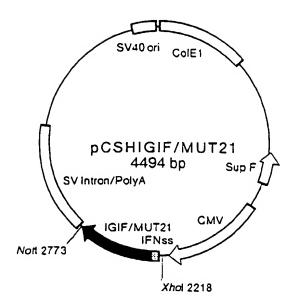


FIG. 4

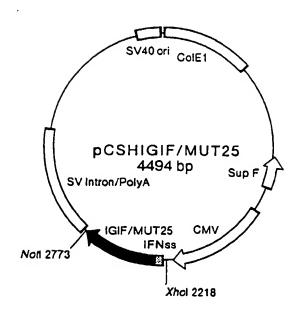


FIG. 5

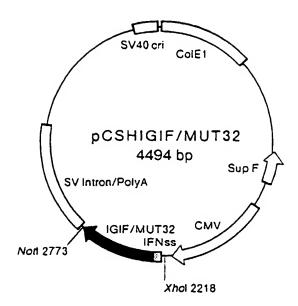


FIG. 6

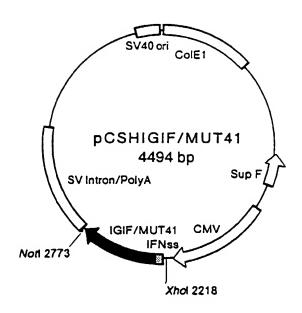


FIG. 7

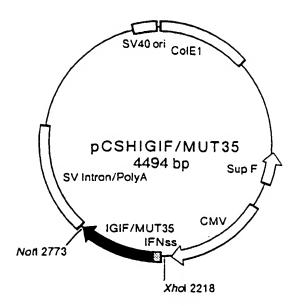


FIG. 8

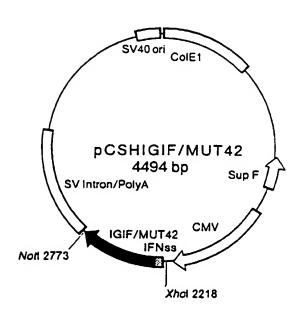


FIG. 9

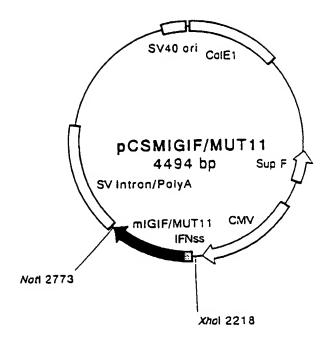


FIG. 10

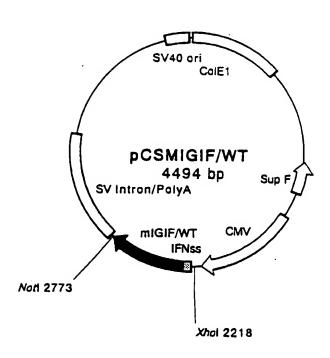


FIG. 11

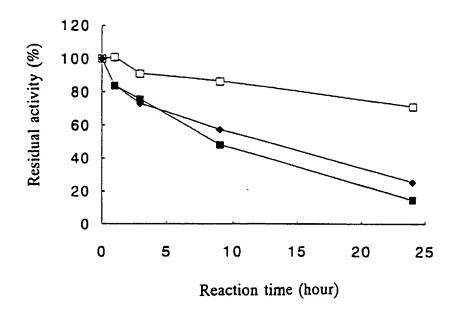


FIG. 12

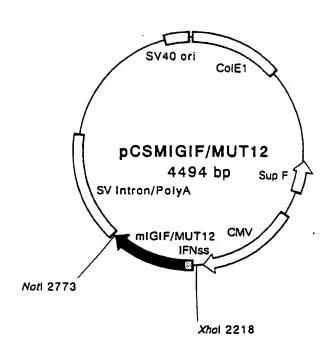


FIG. 13

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